UNIVERSIDADE DE LISBOA
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Functioning of coastal food webs – intertidal rock pools as a case-study

"Documento Definitivo"

Doutoramento em Biologia

Especialidade de Biologia Marinha e Aquacultura

Vanessa Sofia Alegria Mendonça

Tese orientada por:
Professora Doutora Catarina Vinagre
Professor Doutor Augusto Flores

Documento especialmente elaborado para a obtenção do grau de doutor

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Júri:
Presidente:

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● Doutora Catarina Maria Batista Vinagre, Professora Auxiliar Convidada, Faculdade de Ciências da Universidade de Lisboa (Orientadora)

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Nota prévia

A presente tese apresenta artigos científicos já publicados ou submetidos para publicação (capítulos 2 a 6), de acordo com o previsto no n˚2 do artigo 25˚ do Regulamento de Estudos de Pós Graduação da Universidade de Lisboa, publicado em Diário da República 2ª série – N˚155 – 11 de Agosto de 2017. Uma vez que estes trabalhos foram realizados em colaboração, a candidata esclarece que participou integralmente na conceção dos trabalhos, obtenção de dados, análise e discussão dos resultados, bem como na redação dos manuscritos.

A presente tese está redigida em Inglês por ser uma compilação de publicações internacionais. No final de cada capítulo é dada uma lista de referências e devido a este formado pode haver duplicação entre capítulos. No fim da tese encontra-se o material de suporte de cada capítulo.

Lisboa, Outubro de 2019

Vanessa Mendonça

“Nas grandes batalhas da vida, o primeiro passo para a vitória é o desejo de vencer!”

Mahatma Gandhi

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ABSTRACT

Rocky intertidal ecosystems have long attracted the attention of biologists, being viewed as natural laboratories, where biodiversity and species-interactions could be easily investigated. However, intertidal rock pools have received much less attention that the surrounding emergent intertidal platforms. The aim of this thesis was to provide new insights into the trophic ecology of intertidal rock pools through 1) the characterization of pools as proxies for the study of marine food web networks; 2) the investigation of food web network robustness to species loss in temperate and tropical ecosystems; 3) the investigation of food web robustness to heat-waves in temperate and tropical ecosystems; 4) the description of seasonality in the food web networks; and 5) the testing of the role of pools as preferential feeding grounds for transient fish species. It was concluded that intertidal rock pools can be used as proxies for the study of marine food web networks. Tropical food webs revealed higher robustness than temperate food webs, however the tropical food webs' topology suffered more alterations after species loss. Food web networks presented similar robustness to the removal of species based on thermal vulnerability, however the tropical webs encompass more thermally vulnerable species and should, thus, suffer more species loss in a heat-wave context. The basic topology of temperate webs was stable throughout the year. Intertidal rock pools are likely used as preferential feeding grounds by early-stages of transient fish, as showed by the consistent similarity between stomach contents and prey availability inside the pool, observed for all species. Overall, this work opens a brand-new research avenue with the use of intertidal rock pools as proxies for the study of marine food web networks, while also shedding light into the particular vulnerability of tropical food webs and the important role of pools in fish early-life.

Keywords: tide pools, network structure, robustness, seasonality, feeding grounds

RESUMO

Os ecossistemas compreendem um grande número de organismos que interagem e dependem uns dos outros. O ambiente marinho enfrenta, nos dias de hoje, um enorme declínio na biodiversidade e no habitat natural disponível, um aumento de espécies invasoras, mudanças climáticas e outras alterações causadas pelos impactos humanos. As alterações que afetam as espécies marinhas, também provocam um impacto significativo nos bens e serviços fornecidos pelo oceano à sociedade humana, cujas consequências são até agora em grande parte desconhecidas. Num mundo em que as mudanças climáticas são cada vez mais evidentes, grandes desafios vão ser enfrentados nas próximas décadas para proteger a biodiversidade, particularmente nos trópicos onde a vulnerabilidade às mudanças climáticas é mais alta e as medidas de proteção mais escassas. Um dos objetivos principais da ecologia, na atualidade, é entender os mecanismos que geram e mantêm a biodiversidade nos ecossistemas, mas a complexidade dos sistemas biológicos dificulta essas análises. As redes tróficas descrevem de forma simplificada as interações alimentares entre as espécies. A estrutura das redes alimentares pode ser quantificada, analisada e modelada, permitindo caracterizações e comparações da topologia das redes alimentares entre comunidades totalmente diferentes e independentemente da identidade das espécies. A partir das propriedades das redes alimentares podem ser calculados modelos que permitem analisar a robustez da rede e prever a sua sensibilidade a distúrbios.

A análise de redes alimentares complexas em ecossistemas abertos apresenta altos custos e desafios logísticos insuperáveis. Dessa forma, esta tese teve como ponto de partida a necessidade de encontrar um sistema de dimensão menor que pudesse ser usado como modelo para o estudo das redes alimentares. Foram selecionadas as poças de maré por serem microcosmos permanentemente imersos, com limites definidos, que abrigam uma elevada biodiversidade, são acessíveis e de fácil manipulação. Há muito que os ecossistemas rochosos da zona entre-marés atraem a atenção dos biólogos, por serem laboratórios naturais onde o estudo da biodiversidade e da interação entre espécies é facilitada pelo fácil acesso. No entanto, as poças de maré receberam muito menos atenção do que os habitats de plataforma rochosa.

Desta forma, o primeiro trabalho desta tese responde à questão “As poças de maré têm potencial de serem usadas como representantes de ecossistemas maiores para a pesquisa de redes alimentares complexas?” Sendo a resposta positiva, colocam-se as seguintes perguntas, nos trabalhos que se seguem: “Quais são os ecossistemas mais vulneráveis à perda de espécies, os ecossistemas temperados ou os tropicais?”, “Quais são as características das espécies associadas ao risco de extinção secundária?”, ”Quais são as consequências para a estrutura das redes

alimentares quando as espécies vulneráveis ao aquecimento são removidas?", "As redes alimentares tropicais são mais vulneráveis ao aquecimento do que as redes alimentares temperadas?", "As propriedades das redes alimentares, estimadas a partir de amostras de biodiversidade das poças naturais, variam sazonalmente em regiões temperadas?" e "Os juvenis de peixes marinhos usam as poças de maré como áreas preferenciais de alimentação?".

Os locais de estudo compreenderam ecossistemas da zona de entre-marés, localizados em diferentes latitudes e ecoregiões do mundo: Mar Celta (Reino Unido, 50ºN), Golfo de São Lourenço (Canadá, 48ºN), Costa sudoeste da Europa (Portugal, 38ºN), Ilha da Madeira (Portugal, 32ºN), Nordeste do Brasil (Brasil, 3ºS) e sudeste do Brazil (Brazil, 23ºS). Um número muito elevado de poças de maré foi analisado (n = 116) de forma a obter resultados conclusivos. Todos estes locais de estudo foram usados para dar resposta à primeira questão da tese. As várias questões relacionadas com a robustez da rede trófica e comparação entre ecossistemas temperados e tropicais, foram testadas em Portugal e no sudeste do Brasil. A sazonalidade das redes tróficas foi testada Portugal, enquanto que o papel das poças como local preferencial de alimentação por peixes não-residentes foi testado no sudeste do Brasil. A escolha dos locais para cada um dos estudos prendeu-se com questões relacionadas sobretudo com a existência de estudos prévios a nível local que garantiam a existência de informação crucial para o estudo (e.g. dados sobre os limites térmicos superiores das espécies ocorrentes nas poças) ou com a existência de infraestrutura de apoio aos trabalhos específicos em curso.

As metodologias gerais para atingir os objetivos incluíram recolha e identificação de organismos em poças de maré dos vários locais de estudo, construção de matrizes das relações tróficas entre os organismos, construção de modelos de redes alimentares altamente resolvidas, análise e modelação computacional das redes tróficas recorrendo ao *software* Network3D para avaliação da robustez e das propriedades das redes, e analise estatística de todos os conjuntos de dados gerados. Para a avaliação da importância das poças de maré na alimentação dos peixes juvenis intertidais as metodologias incluíram a recolha e identificação de peixes transientes em poças de maré tropicais e dos organismos que ocorrem dentro das poças, na zona entre-marés e na zona subtidal adjacente às poças, a caracterização da sua dieta, a análise da correlação da composição específica da dieta com a composição das amostras provenientes do interior da poça, da zona entre-marés e da zona subtidal adjacente à poça. Por fim, todos os dados foram analisados estatisticamente de forma a determinar o grau de semelhança entre a dieta e as amostras dos vários locais de alimentação alternativos.

A presente tese é composta por sete capítulos: o capítulo 1 é uma breve introdução ao tema das redes alimentares e às características das poças de maré escolhidas como objeto de estudo, bem como uma revisão de literatura, enquadramento dos trabalhos realizados, e uma descrição da estrutura da presente tese. O capítulo 2 é o ponto de partida para a escolha das poças de maré como modelos para o estudo das redes alimentares; nos capítulos 3 e 4, é analisada a vulnerabilidade dos ecossistemas temperados e tropicais à perda de espécies e ao aquecimento climático, do ponto de vista da robustez das redes alimentares; no capítulo 5, é investigada a variabilidade sazonal das propriedades das redes alimentares dos ecossistemas temperados; no capítulo 6 é analisada a importância das poças de maré da zona tropical na alimentação de peixes juvenis transientes. Por fim, no capítulo 7, são apresentadas as considerações finais e as perspetivas futuras.

Com este trabalho foi possível concluir que: i) as redes alimentares das poças de maré compartilham características organizacionais fundamentais com as redes alimentares de ecossistemas diferentes, maiores, abertos e abioticamente mais estáveis, e por isso podem ser usadas como modelos para a compreensão dos processos universais que regulam a complexa organização das redes alimentares; ii) as redes alimentares tropicais apresentaram maior robustez que as redes alimentares temperadas, no entanto, a topologia das teias alimentares tropicais sofre mais alterações após a perda de espécies. As teias alimentares temperadas e tropicais são menos robustas quando a remoção é direcionada às espécies mais conectadas, confirmando que espécies altamente conectadas são particularmente importantes nas redes alimentares; iii) as teias temperadas e tropicais apresentam robustez estrutural semelhante quando a remoção de espécies é feita com base na vulnerabilidade térmica, mas as redes alimentares tropicais sofrerão mais alterações na rede alimentar num contexto de onda-de-calor porque incluem mais espécies vulneráveis a temperaturas elevadas; iv) não foram observadas variações sazonais na estrutura da rede alimentar, não havendo variação na proporção de espécies de nível superior, intermédio e basal, bem como na proporção de espécies herbívoras, omnívoras e canibais, revelando que a topologia básica das redes se mantém estável ao longo do ano; v) as poças de maré são provavelmente usadas como áreas de alimentação pelos juvenis de espécies de peixes não-residentes na poça, devido à similaridade encontrada entre o conteúdo estomacal e a disponibilidade de presas dentro das poças de maré observada para todas as espécies. Este trabalho abre uma linha de pesquisa inteiramente nova ao descrever a topologia básica das redes alimentares das poças de maré, concluindo sobre a sua utilidade como ecossistema-modelo para o estudo de teias tróficas marinhas, alertando também para a particular vulnerabilidade dos ecossistemas tropicais e testando o importante papel das poças-de-maré como locais preferenciais de alimentação de juvenis de peixes não-residentes em poça.

Palavras-chave: poças de maré, redes alimentares, robustez, sazonalidade, áreas de alimentação

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA, analysis of variance

AVG, average

BEST, biotic patterns to environmental variables

CE, Ceará

CEBIMar, Centro de Biologia Marinha

CEUA USP, Comissão de Ética no uso de Animais da Universidade de São Paulo

CEUA, Comissão de Ética na Utilização de Animais

cm, centimeter

DGAV, Direcção Geral de Veterinária

E, east

e.g., for exemple

FCT, Fundação para a Ciência e Tecnologia

Fig., figure

g, grams

i.e., in other words

IPCC, Intergovernmental Panel on Climate Change

Lt, Total length

m, metres

m^2, square meter

MARE, Marine and Environmental Sciences Centre

Max, maximum

mg, milligrams

MHT, Maximum Habitat Temperature

Min, minimum

mL, mililitre

mm, milimetres

N, north

n, total number

NE US, Northeastern United States

º, degree

p, p-value

PERMANOVA, permutational multivariate analysis of variance

pH, power of hydrogen

PhD,

S, south

SD, standard deviation

SIMPER, analysis of similarity

SP, São Paulo

SST, sea surface temperature

UK, United Kingdom

US, United States

USA, United States of America

USP, Universidade São Paulo

W, west

Wt, weight

Σ, summation

%, percentage

~, approximately

˚C, degree Celsius

<, less

>, greater

±, more or less

| |, absolute value

‰, per mil

CHAPTER 1

General introduction & literature review

1. General introduction & literature review

1.1. Intertidal rock pools

A rocky shore is an intertidal area where the predominant substrate is composed of solid rocks. It is often a biologically rich environment that can include a great variety of habitats with varying heterogeneity, such as cliffs, platforms, boulder fields and intertidal rock pools (Underwood, 1984; Menge et al., 1995; Sebens, 1991; Johnson et al., 2003; Kostylev et al., 2005).

Intertidal organisms are exposed to a challenging environment with successive periods of immersion and emersion due to the tidal cycle (Widdows & Brinsley, 2002). Worldwide studies have described the general structure and organization of rocky shore biological communities, and an extensive literature has been synthesised by Paine (1994), Little & Kitching (1996), Raffaelli & Hawkins (1996) and Hawkins et al. (2019). Classical descriptive works include descriptions of patterns of biological distribution (e.g., Lewis, 1964, Brattstrom, 1980; Norton, 1985; Russel, 1991; Boaventura et al., 2002a; Pereira et al., 2006), the universal zonation scheme (Stephenson & Stephenson, 1949, 1972; Lewis, 1961, 1964; Pérès & Picard, 1964) and the effect of physical and biological factors on marine intertidal communities (e.g. Southward, 1958; Ballantine, 1961; Connell, 1972; Underwood, 1981; McQuaid & Branch, 1984; Hawkins & Hartnoll, 1985; Benedetti-Cecchi, et al., 2000).

Experimental studies aiming to understand the ecological functioning of rocky shores include the analyses of disturbance and succession on intertidal communities (e.g. Dayton, 1971; Sousa, 1979, 1984; Dethier, 1984; Farrell, 1991; Benedetti-Cecchi & Cinelli, 1993; McCook & Chapman, 1997; Cruz-Motta et al., 2010; Flores & Paula, 2001; Araújo et al., 2005), and experimental studies on competition (Connell, 1961; Paine 1974; Branch, 1976; Lubchenco & Menge, 1978; Underwood, 1978, 1984b; Boaventura et al., 2002b; Caro et al., 2011), grazing (Lubchenco, 1978, 1980; Underwood, 1980; Hawkins, 1981; Hawkins & Hartnoll, 1983; Benedetti-Cecchi et al., 1996; Jenkins et al., 1999a, b; Boaventura, 2000; Coleman et al., 2006), predation (Paine, 1966; Menge, 1976; 1978a, 1978b; Menge & Lubchenco, 1981; Silva et al., 2004; Brazão, et al., 2009) and recruitment (Denley & Underwoord, 1979; Hawkins & Hartnoll, 1982; Menge, 1991; Jenkins et al., 1999c; Bulleri, 2005). It is, thus, a well-studied environment.

Intertidal rock pools are patchy depressions which retain seawater during low tide and occur on rocky shores across the world, however, their biological communities are less well studied than the emergent substrata of rocky intertidal environments (but see Metaxas &

Scheibling, 1993; Underwood & Skilleter, 1996; van Tamelen, 1996; O'Connor & Crowe, 2005; Martins et al., 2007; Firth et al., 2014).

The conditions in intertidal rock pools are regulated by the tidal cycle. Physical conditions vary horizontally, vertically, daily and seasonally (Metaxas & Scheibling, 1993; Knox, 2000). The amplitude of fluctuation is lower than that occuring on the emergent substrata and larger than that occuring on the subtidal zone (Metaxas & Scheibling, 1993; Knox, 2000). These fluctuations in physical conditions (temperature, salinity, pH, oxygen saturation and alkalinity) depend on the volume, depth and surface area of the pool, as well as on its degree of shading, drainage pattern, height on the shore, and exposure to waves and ocean splash (Klugh, 1924; Lami, 1931; Stephenson et al., 1934; Pyefinch, 1943; McGregor, 1965; Ganning, 1971; Green, 1971; Daniel & Boyden, 1975; Goss-Custard et al., 1979; Morris & Taylor, 1983; Huggett & Griffiths, 1986; Legrand et al., 2018), making every pool unique in their physical regime. Intertidal rock pools placed lower on the shore exhibit less variability in environmental conditions than pools located in the upper shore that are under longer emersion periods (Pyefinch, 1943; Huggett & Griffiths, 1986).

Pools of lower volume have more extreme environments because they have less water to mitigate alterations in abiotic conditions (Ganning, 1971; Metaxas & Scheibling, 1994; Therriault & Kolasa, 2000; Firth & Williams, 2009). Area has little effect on the biological communities inhabiting pools (Underwood & Skilleter, 1996; Martins et al., 2007), depth influences species diversity and community composition (Kooistra et al., 1989; Astles, 1993; van Tamelen, 1996; Moschella et al., 2005; Bussell et al., 2007; Martins et al., 2007; Browne & Chapman, 2014; Firth et al., 2014) but little is known on the effect of inclination (Johnson & Skutch, 1928). Various works have shown that deeper pools support more plant, invertebrates and fish (Droop, 1953; Pajunen, 1977; Ranta, 1982; Fairweather & Underwood, 1991; Marsh et al., 1978; Bennett & Griffiths, 1984; Mgaya, 1992).

The organisms that reside in pools remain submerged for the entire tidal cycle. Although there is little evidence in the literature it is accepted that intertidal rock pools often support greater diversity, abundance and/or biomass of organisms than emergent rock (Goss-Custard et al., 1979; Chapman & Johnson, 1990; Firth et al., 2013a). For example, species like algae and gastropods are more abundant in pools, while others are absent or fewer than on adjacent open rocks (Metaxas & Scheibling, 1993). The high productivity of pools is mostly explained by the diversity of invertebrates (Ganning, 1971; Firth et al., 2014) and seaweeds (Araujo et al., 2006)

The type of organisms recorded in intertidal rock pools include macroalgae (e.g. the genera Ulva, Enteromorpha, Cladophora, Ceramium, Spongomorpha, Corallina, Lithothamnion and Rhizoclonium) (Ganning, 1971; Lubchenco, 1982; Kooistra et al., 1989; Metaxas & Scheibling, 1993), phytoplankton (Droop, 1953; Metaxas & Lewis, 1992), zooplankton (Metaxas & Scheibling, 1993), meiofauna species (mostly composed of flatworms, oligochaetes, rotifers, cladocerans, ostracods, copepods, barnacles, isopods and amphipods) (Dethier, 1980, 1984; Metaxas & Lewis, 1992), gastropods (e.g. limpets, chitons, whelks) (Underwood, 1976; Metaxas et al., 1994; Esqueda et al., 2000), bivalves (e.g. mussels) (Esqueda et al., 2000; Metaxas et al., 1994), sea urchins (Metaxas et al., 1994), crabs (Cannicci et al., 1999; Flores & Paula, 2001), shrimps (Amara & Paul, 2003; Vinagre et al., 2015) and fishes (Gibson, 1982; Bennett & Griffiths, 1984; Amara & Paul, 2003; Almada & Faria, 2004; Barreiros et al., 2004; Dias et al., 2014; Mendonça et al., 2018).

For a wide range of organisms the topographic heterogeneity of pool's substrata (vegetation cover, debris and substratum rugosity) provides important physical refuge zones (Schonbeck & Norton, 1978; Underwood & Jernakoff, 1984; Moran, 1985; Fairweather, 1988), refuge from herbivory (Lubchenco, 1983; Menge et al., 1985), refuge from predation (McGuinness & Underwood, 1986) and refuge from desiccation (Menge et al., 1985; Fairweather, 1988; Gosselin & Bourget, 1989). Pools' substrata also provide nursery grounds (Orton, 1929; Norris, 1963; Lewis & Bowman, 1975; Thompson, 1980; Bennett, 1987; Delany et al., 1998; Dias et al., 2014); and feeding habitat for many species (Thompson & Lehner, 1976; Moring, 1990; Wai & Williams, 2006a, b; Noël et al., 2009).

In intertidal rock pools there are strong interactions between biological processes and physico-chemical parameters (Benedetti-Cecchi et al., 2000; Underwood & Jernakoff, 1984; Legrand et al., 2018), i.e. abundance and species distribution are controlled by abiotic factors and species interactions, such as competition, predation and herbivory (Truchot & Duhamel-Jouve, 1980; Huggett & Griffiths, 1986; Metaxas & Scheibling, 1993).

Some species tend to aggregate in pools, while others avoid them, however intertidal rock pools can extend the upper vertical limits of many organisms susceptible to desiccation (Johnson & Skutch, 1928; Goss-Custard et al., 1979; Metaxas & Scheibling, 1993; Araujo et al., 2006; Firth & Crowe, 2008, 2010; Firth et al., 2013a). Although pools can offer protection from the challenging conditions on the emergent substrata (e.g. temperature and desiccation), they can also exhibit large amplitudes in salinity, temperature, carbon dioxide, pH and dissolved oxygen, and, thus, turn into stressful environments (Goss-Custard et al., 1979; Metaxas &

Scheibling, 1994; Chan, 2000; Firth & Williams, 2009; Legrand et al., 2018). Intertidal rock pools are among the habitats where the effects of warming will be felt most quickly. They present lower thermal inertia than their surrounding waters, reaching very high temperatures during heat waves (Helmuth et al., 2006; Vinagre et al., 2016). Therefore, they are considered particularly sensitive to warming and it has been shown that they can work as ecological traps for coastal organisms, especially in the tropics, because they can reach temperatures higher than the upper thermal limits of numerous intertidal species, increasing local extinction risk (Vinagre et al., 2018).

Several studies analysed the role of intertidal rock pools as nursery grounds for juvenile marine fish and shrimp (Thompson & Lehner, 1976; Beckley, 1985a; Bennett, 1987; Moring, 1986; Gibson, 1994; Santos et al., 1994; Mahon & Mahon, 1994; Macpherson, 1998; Gibson & Yoshiyama, 1999; Strydom, 2008; Vinagre et al., 2015; Dias et al., 2014, 2016). It is generally agreed that this occurs because pools provide refuge from predators (Bennett & Griffiths, 1984; Davis, 2000), favourable temperatures for growth (Gibson, 1994) and high food availability, which makes them preferential feeding grounds (Beckley, 1985a; Moring, 1986; Amara & Paul, 2003; Cunha et al., 2007).

Intertidal rock pools are natural microcosms useful for the study of the mechanisms that generate community patterns and structure, since organisms can be easily manipulated and the access to this habitat is easily granted (Dayton, 1971; Paine, 1974; Menge, 1976, 1995; Lubchenco, 1978). Also, since the impacts of climate warming will apparently be higher on these habitats they are considered natural laboratories, not only for the study of community dynamics but also for climate change research (Helmuth et al., 2006; Menge, 2008; Firth et al., 2009; Hawkins et al., 2009; Rastrick et al., 2014; Morley et al., 2016; Firth et al., 2016; Vinagre et al., 2018, 2019). For all this, intertidal rock pools are interesting model-ecosystems.

1.2. Food webs

1.2.1. Concepts and data

A food web is an important ecological concept, representing the feeding relationships within a biological community (Smith & Smith 2009). Food web studies have historically been based on two approaches: the approach proposed by Lindeman (1942), depicting an energetic view of the food webs as networks of pathways of energy flow; and the topological approach proposed by May (1973) and Pimm (1982), focusing on the dynamic constraints that arise from species interactions. This last approach allowed many comparative works on the topology of food webs (e.g. Briand & Cohen, 1984; Hall & Rafaelli, 1991). More, recently studies on food web structure have focused on food web network theory – a field of research that integrates food web ecology and complex networks (e.g. Williams & Martinez 2000, 2004; Dunne et al., 2002a, b, 2004). Using the language of network analysis, species are represented by vertices (nodes) and feeding links are represented by edges (links) between vertices. The present work follows this line of research.

To build a food web network, the data on trophic relationships provided by the literature are transformed into binary matrices with n rows and m columns. Each column is headed by the number of the resource taxa and each row is headed by the number of the consumer taxa. Inside the matrix, the presence or absence of a feeding link between a consumer and a resource is represented by 1s or 0s, it equals 1 if the consumer *j* eats resource *i* or 0 if *j* does not eat *i* (Fig. 1.1 a). Analysis software recognizes a two-column format where a consumer's number appears in the first column, and its resource's numbers appears in the second column (Fig. 1.1 b).

a

Prey Pred	1	2	3	4
1	0	0	0	0
2	1	1	0	0
3	1	1	1	0
4	1	0	1	0

b

Pred	Prey
2	1
2	2
3	1
3	2
3	3
4	1
4	3

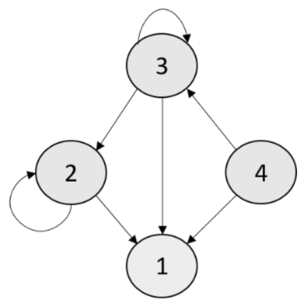

Figure 1.1. Example of hypothetical food web with 4 taxa and 7 links. Numbers 1–4 correspond to the different taxa, a matrix format: the 1s or 0s inside the matrix denote the presence or absence of a feeding link between a consumer (Pred) and a resource (Prey); b Two-column format: a consumer's (Pred) number appears in the first column, and one of its resource's (Prey) numbers appears in the second column; In this hypothetical web, taxa numbers 1 are basal taxa (i. e., taxa that do not feed on other taxa—autotrophs or detritus), and taxa numbers 2 and 3 have cannibalistic links to themselves. Author: V. Mendonça, 2019

The structure of food webs is defined by network properties ranging from typical ecological-community variables (i.e. trophic level, percentage of basal species, percentage of top species) to general graph theory metrics (i.e. connectance, characteristic path length, clustering coefficient) (Dunne et al., 2002b, 2004; Riede et al., 2010). The structure of these food web networks can be quantified, analysed and mathematically modelled. This has enabled the characterisation and comparison of food web topology among communities and other types of networks and the search for universal organization features of food webs irrespective of species' identities (e.g. Williams & Martinez, 2000; Solé & Montoya, 2001; Dunne et al., 2002b; Dunne, 2006; Montoya et al., 2006; Bascompte, 2009; Mendonça & Vinagre, 2018). Network properties also allow the analyses of community resilience, stability, functionality (Dunne et al., 2002a; Williams et al., 2002; Montoya et al., 2006; Otto et al., 2007; Tylianakis et al., 2010; Stouffer & Bascompte, 2010) and robustness against secondary extinctions (Dunne et al,. 2002a; Staniczenko et al., 2010; Gravel et al., 2011; Curtsdotter et al., 2011; Riede et al., 2011). This way, network properties allow not only the comparison of ecological structure among ecosystems, but also to predict their susceptibility to disturbances.

1.2.2. General differences between food webs and other networks

The growing amount of studies on network structure has revealed differences between food webs and other networks: food webs have identical path lengths but a lower level of clustering and have exponential or even uniform degree distributions, i.e. the links are more uniformly distributed throughout the network (Dunne et al., 2002b). Other networks like social networks or biological networks (e.g. gene expression networks or protein network) share a similar small-world topology with high clustering and power-law degree distributions, but small path lengths (Watts & Strogatz, 1998; Albert & Barabási, 2002).

Notwithstanding, the differences between food webs networks and networks of different nature, there is a great interest in the analysis of the topology of food webs (Dunne et al., 2004; Dunne et al., 2008; Riede et al., 2010) since topological properties allow the comparison of completely different systems (e.g. Riede et al., 2010). Some topological generalities have been revealed, providing insight into the ecological processes structuring the community. Comparison of the topology of different ecosystems has revealed only small differences in the food web network properties. Most properties display scaling relations with species richness and connectance and follow scaling laws independent of ecosystem differences (Dunne et al., 2004; Dunne et al., 2008; Riede et al., 2010).

The analyses of network structure in the present work are purely topological since they only consider link structure of the networks and do not take into account the growth rates and dynamics of populations. Recent theoretical advances in dynamical food web models have shown the importance of body mass, degree and allometric degree distributions (Woodward et al., 2005; Montoya et al., 2006; Otto et al., 2007; Berlow et al., 2008). The concept of degree distributions extended to allometric degree distributions describes the relationship of indegree (generality) or outdegree (vulnerability) in dependence of the species body mass, irrespective of their taxonomy or other traits (Otto et al., 2007).

The species body size generally determines its trophic position in the food web and has implications on the physiological and ecological characteristics of species (Jennings et al., 2001; Woodward & Hildrew, 2002; Cohen et al., 2003; Woodward et al., 2005). Recent studies have shown that some properties of food webs are correlated with species body sizes, so large predators tend to have more prey species and small prey species tend to have more predators (Brose et al., 2006; Otto et al., 2007; Brose, 2010).

Such allometric degree distributions stabilize the dynamics of the biological community and, in robustness analysis, prevent extinctions from occurring (Otto et al., 2007). Dynamic approaches are very useful, however in most ecosystems abundance and interaction strength are unknown for the vast majority of species. Thus, although allometric degree distributions are a promising research topic to explain diversity and stability of ecosystems a topological approach is oftentimes the only possible approach.

A significant feature of food web network structure (topology) analysis is that it can be done for any food web, irrespective of additional data availability (such as body size, body mass, abundance or energy flux magnitude through the trophic links) because they are based exclusively on the presence/absence of taxa and feeding links within a web (Paine, 1988).

1.2.3. Food web properties

Food webs have been characterized by a variety of topological properties that can be calculated for any network. Many of these properties are quantifiable just by using the basic network structure (topology) of feeding interactions and, as such, have been used to evaluate simple models of food web structure (Dunne, 2012). Examples of food web network structure properties, used in this work, follow.

Fundamental properties

Properties that characterize very simple, overall attributes of food web network structure.

- **Number of species (S) -** Number of nodes in the food web (number of taxa or number of trophic species)
- **Links per species (L/S) -** Number of pred/prey links per species
- **Connectance (C) -** Proportion of actual links to all possible links (L/S^2)

Types of taxa

Properties that characterize the proportion or percentage of taxa within a food web.

- **Top taxa (T) -** Species with prey and without predators or parasites
- **Intermediate taxa (I) -** Species with both predators and prey
- **Basal taxa (B) -** Species with predators and without prey
- **Herbivores plus detritivores (H) -** Species who prey on primary producers or detritus
- **Cannibals (Can) -** Species which prey on their own species
- **Omnivores (Omn) -** Species with food chains of different lengths, where a food chain is a linked path from a nonbasal to a basal species

Network structure

Properties that characterize other attributes of network structure, based on how links are distributed among taxa.

- **Trophic level (TL) -** Trophic level averaged across taxa
- **Mean food chain length (Chain) -** Mean number of links in every possible food chain or sequence of links connecting top species to basal species
- **Mean shortest path length (Path) -** The mean shortest set of links between species pairs
- **Generality standard deviation (GenSD) -** Resources per taxon, how many prey items a species has
- **Vulnerability standard deviation (VulSD) -** Consumers per taxon, how many predators a species has
- **Normalized standard deviation of links (LinkSD) -** Links per taxon

- **Clustering coefficient (Clust) -** The mean shortest set of links between species pairs

1.2.4. Models of food web structure

Various models combining stochastic elements with simple link assignment rules have been proposed in order to generate and predict the network structure of empirical food webs, (Dunne, 2012). These models share a basic formulation, use two empirically quantifiable parameters: S, the number of nodes in a network, and C, the connectance of a food web defined as number of links in a network with S nodes. Each species is assigned a "niche value" n_i drawn randomly and uniformly from the interval [0,1]. The models differ in the rules used to distribute links among species.

In the niche model, proposed by Williams & Martinez (2000), each species is given a uniformly random niche value (n_i) along a segment of the [0,1], and that niche value corresponds to the position of each species on the segment. To distribute links, each species is assigned a feeding range that represents an interval of the line whose midpoint (c_i) is a uniformly random number less than the niche value of the species possessing the range. All species that fall in this segment interval, whose size r_i are drawn randomly from a beta distribution to produce a C close to the target C, are eaten by the consumer species (Fig 1.2).

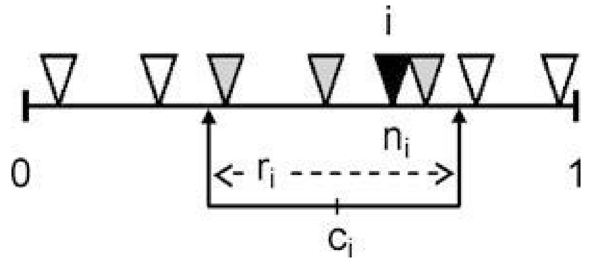

Figure 1.2. Graphical representation of the niche model: species I feeds on four taxa including itself and one with a higher niche value (n_i). Source: Dunne J.A., 2012

The distribution of feeding links in the niche model allows looping, cannibalism and feeding on species with higher niche values; since species with similar niche values are more likely to have the same consumers there is higher trophic overlap; and food webs are rendered interval due to contiguous feeding by each consumer within a single segment of the line, this is an acknowledged limitation of the niche model (Williams & Martinez, 2000). However, Williams & Martinez (2000) argued that intervality is a delicate property that is easily broken by the loss of just one link in a web.

The niche model analysis begins with Monte Carlo simulations to generate hundreds to thousands of model-webs with the same S and C as the empirical web. Then model means and standard deviations are estimated for each food web property and compared to empirical values. The difference between the value of an empirical property and a model mean for that property (Raw error) is normalized by dividing it by the standard deviation (SD) of the model property. The model is considered to be a good fit to the empirical data when the normalized errors are within ± 1 (Williams & Martinez, 2000). This approach allows the assessment of to what degree a model's mean deviates from the empirical value and whether a model over- or under-estimates empirical properties as indicated by the raw error.

1.2.5. Structural robustness of food webs

The relation between food web network structure and their ability to deal with perturbation is a central theme in ecology. Simulated species deletions have been used to examine the reliability of network flow, or the probability that producers are connected to consumers in food webs (Jordán & Molnár, 1999). The use of species loss simulations enables the assessment of potential secondary extinctions in complex food webs, and so evaluate the robustness of ecosystems (Jordán & Molnár, 1999; Solé & Montoya, 2001; Dunne et al., 2002b, 2004; Memmott et al., 2004; Allesina & Bodini, 2004). Secondary extinctions occur when one or more consumers lose all of their prey taxa (Dunne, 2012).

Food webs show similar patterns of robustness:

- removal of highly connected species results in much higher rates of secondary extinctions than random species loss (Solé & Montoya, 2001; Dunne et al., 2002a, 2004);

- loss of high-degree species results in more rapid fragmentation of the food web (Solé & Montoya, 2001);

- excluding basal taxa from primary removal increases the robustness of the web (Dunne et al., 2002a);

- removing species with few links generally results in few secondary extinctions (Dunne et al., 2002a);

Beyond the impacts of sequences of species loss in food webs the analysis of food web ‘structural robustness’ has been the target of numerous studies. Structural robustness (R_{50}), defined as the fraction of primary species loss that induces at least 50% total species loss (primary + secondary extinctions), analyzed in various food webs showed that robustness increases logarithmically with increasing connectance (Dunne et al., 2002a, b, 2004).

From a topological perspective, food webs are better protected from species loss when they have densely interconnected taxa, since it takes greater species loss for consumers to lose all of their resource taxa. In a conservation perspective, it is important to identify critical taxa whose removal leads to more secondary extinctions. The greater the number of species that a particular species links, the greater the number extinctions that may result from its' removal.

The potential for secondary extinctions depends on the sequence in which species go extinct, so it is important that studies use realistic natural extinctions sequences, so their findings can help minimize biodiversity loss in naturally changing environments, however this approach is very rare in literature (Srinivasan et al., 2007).

1.2.6. Software - Network3D

The data used in studies of complex networks contains tens to hundreds of functionally distinct nodes and hundreds to thousands of links between them. Due to the difficulty of synthesizing such information, software was created that enables the visualization and analysis of the data, greatly contributing to ecological research in this area (Yoon et al., 2004; Williams, 2010). This is very useful in network structure and functional analysis. Structure depicts which organisms are present in the network and whom they interact with and function relates to the details of the dynamics of network interactions like the magnitude of trophic flow that transit through network links and the functional responses of consumers to the density of their resources (Yoon et al., 2004). The software used in this work was Network3D and was developed by Richard Williams at Microsoft Research (Williams, 2010). The tools of this software let users visualize and model food webs, interactively manipulate the visualization parameters (i.e. rotate the image, delete species, adjust the colors and sizes of nodes and links), perform web analysis, calculate network properties, compute models properties and test network models (Yoon et al., 2004; Williams, 2010). Nodes (species) are presented as spheres and links appear as lines, with the lines starting from the predators and pointing to the prey (see Fig. 1.3 for example).

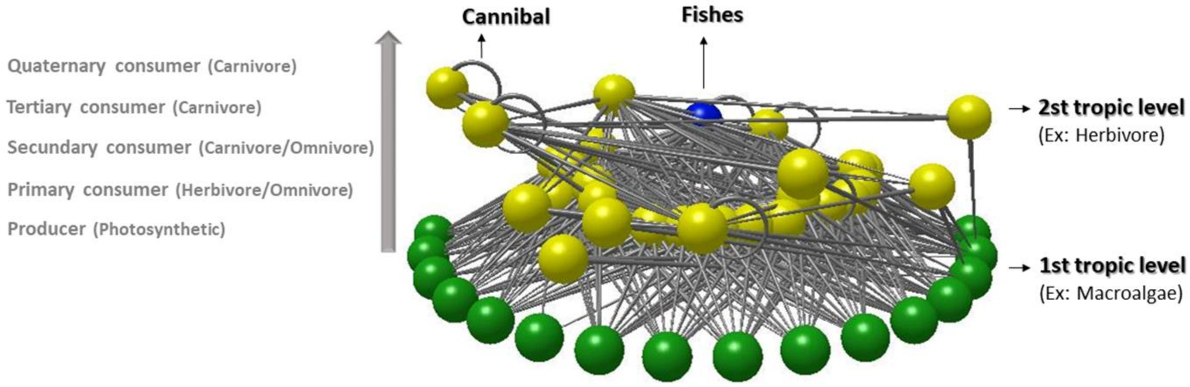

Figure 1.3. Visualization of food web produced with Network3D written by R.J. Williams. Green nodes represent basal species, yellow nodes represent invertebrates and blue nodes represent vertebrates. Author: V. Mendonça, 2019

1.3. Main aims of the thesis

In a world where climate change is increasingly evident, major challenges will be faced in the next decades to protect biodiversity, particularly in the tropics where warming is expected to be faster and where many thermally vulnerable organisms have been identified (Vinagre et al., 2019). Knowledge on ecosystem functioning will be crucial to face the future scientific and societal demands, however the knowledge gaps are numerous, hindering an appropriate future response. Understanding food web functioning is central to understanding the fate of biodiversity in response to natural and anthropogenic perturbations. However, the investigation of complex food web dynamics over large ecosystems is complex, costly, and time-consuming. Since for most ecosystems the most basic information on biodiversity and trophic dynamics is missing, it is fundamental to find small systems that can be used as proxies to advance knowledge on food webs. Here, the usefulness of intertidal rock pools as proxies of marine food webs is tested, answering the question:

1. Do intertidal rock pools have the potential to be used as proxies for larger ecosystems for food web networks research?

The positive answer to this question allowed the investigation of the following questions, which bring new insights into marine food web networks and the trophic role of intertidal rock pools:

2. Which are the most vulnerable food webs to species loss, temperate or tropical?

3. What are the species' traits associated with secondary extinction risk?

4. What are the consequences for the structure of food webs, when species vulnerable to warming are removed?

5. Are tropical food webs more vulnerable to warming than temperate food webs?

6. Do food web properties, estimated from intertidal rock pool biodiversity samples, vary seasonally in temperate regions?

7. Do marine fish juveniles use intertidal rock pools as feeding grounds?

To answer the first question, an important number of study sites, were selected for sampling, located at different latitudes and ecoregions of the world: Celtic Sea (United Kingdom, 50ºN), Gulf of Saint Lawrence (Canada, 48ºN), South European Atlantic shelf (Portugal, 38ºN), Madeira Island (Portugal, 32ºN), Northeast Brazil (Brazil, 3ºS), Southeast Brazil (Brazil, 23ºS), in a large-scale sampling effort covering 116 intertidal rock pools.

The robustness of the food webs and the comparison between temperate and tropical ecosystems were tested in Portugal and southeast Brazil. Seasonality of food webs was tested in Portugal and the use intertidal rock pools as feeding grounds for marine fish juveniles was tested in southeast Brazil. The choice of sites for each of the studies was due to the existence of previous studies that ensured crucial information for the study (e.g. data on upper thermal limits of species occurring in the pools), to the existence of on-going collaboration with local experts and/or the existence of support infrastructure for specific work in progress.

In summary, the overall output of this thesis was the characterization of intertidal rock pools as proxies for the study of marine food web networks; the analysis of the robustness of temperate and tropical ecosystems to species loss, the comparison of the robustness of temperate and tropical food webs to the loss of thermally vulnerable species, the investigation of seasonal variability in food web network structure; and the definition of the importance of intertidal rock pools as feeding grounds for transient fish.

1.4. Thesis outline

Briefly, this thesis (Fig. 1.4) is comprised of 7 chapters. The first chapter is a brief introduction to the theme of food webs and the characteristics of intertidal rock pools chosen as a case-study, as well as, a presentation of the structure of the present thesis; chapter 2 is the starting point for choosing intertidal rock pools as a proxy for the study of food web network structure; in chapters 3 and 4, the vulnerability of temperate and tropical food webs to species loss and climate warming is analyzed, respectively; in chapter 5 it is investigated whether the food web properties vary seasonally in temperate ecosystems; in chapter 6 the importance of intertidal rock pools in the feeding of juvenile transient fish is analysed. The last chapter, Chapter 7, presents the concluding remarks and future perspectives.

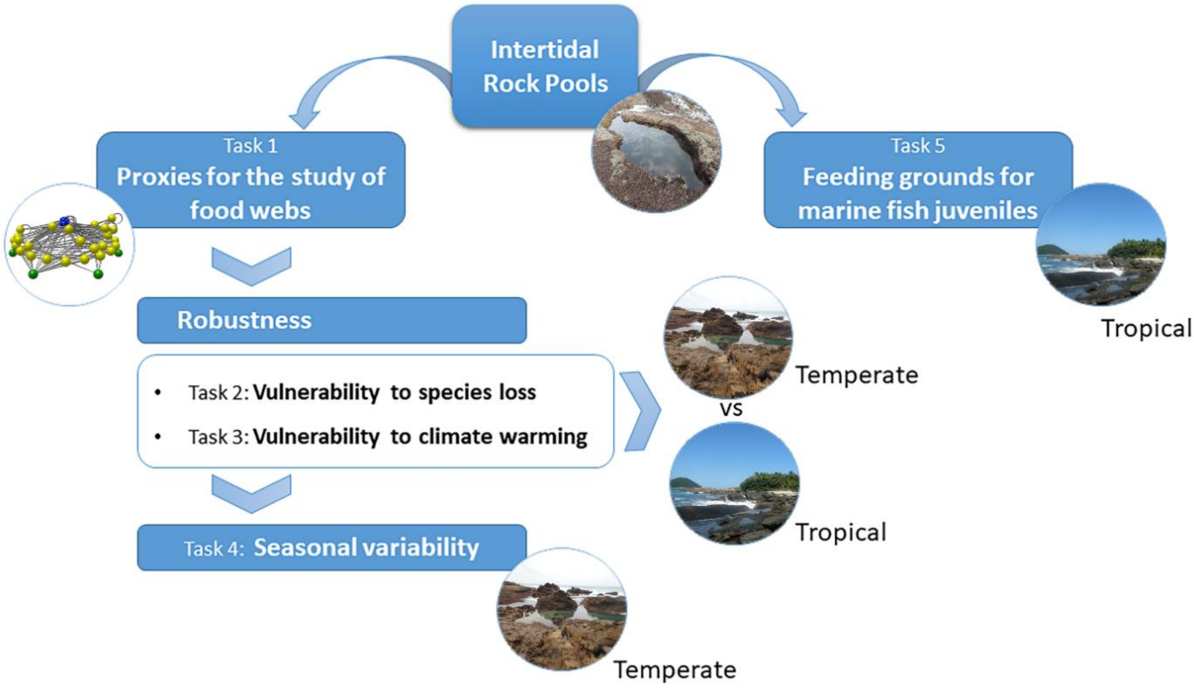

Figure 1.4. PhD workplan flowchart. Author: V. Mendonça, 2019

More specifically, in Chapter 2 the complex network structure of food webs occurring in intertidal rock pools was investigated, for the first time, and compared to the ones of other habitats, by estimating their network properties and fit to the theoretical niche food web model. In Chapter 3 the robustness, a measure of network tolerance to species loss, was estimated for temperate *versus* tropical ecosystems, using five different criteria of sequential deletion (most-connected, least-connected, most-abundant, largest body mass and largest size). In Chapter 4, the robustness of tropical and temperate food web networks to the sequential removal of species according to a ranking of thermal vulnerability previously established experimentally by Vinagre et al. (2018) was analysed. In Chapter 5, the seasonal variation in food web properties, estimated from intertidal rock pool biodiversity samples from the temperate region of the west coast of Portugal, was examined. Finally, in Chapter 6 the diet of a common transient fish species that occurs in intertidal rock pools was characterized and compared with the prey available in the intertidal zone in order to evaluate the importance of intertidal rock pools in the feeding ecology of juvenile transient fish. Chapter 7 presents the final conclusions and future perspectives.

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CHAPTER 2

What's in a tide pool? Just as much food web network complexity as in large open ecosystems

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ABSTRACT

Understanding the fundamental laws that govern complex food web networks over large ecosystems presents high costs and oftentimes unsurmountable logistical challenges. This way, it is crucial to find smaller systems that can be used as proxy food webs. Intertidal rock pool environments harbour particularly high biodiversity over small areas. This study aimed to analyse their food web networks to investigate their potential as proxies of larger ecosystems for food web networks research. Highly resolved food webs were compiled for 116 intertidal rock pools from cold, temperate, subtropical and tropical regions, to ensure a wide representation of environmental variability. The network properties of these food webs were compared to that of estuaries, lakes and rivers, as well as marine and terrestrial ecosystems (46 previously published complex food webs). The intertidal rock pool food webs analysed presented properties that were in the same range as the previously published food webs. The niche model predictive success was remarkably high (73-88%) and similar to that previously found for much larger marine and terrestrial food webs. By using a large-scale sampling effort covering 116 intertidal rock pools in several biogeographic regions, this study showed, for the first time, that intertidal rock pools encompass food webs that share fundamental organizational characteristics with food webs from markedly different, larger, open and abiotically stable ecosystems. As small, self-contained habitats, intertidal rock pools are particularly tractable systems and therefore a large number of food webs can be examined with relatively low sampling effort. This study shows, for the first time that they can be useful models for the understanding of universal processes that regulate the complex network organization of food webs, which are harder or impossible to investigate in larger, open ecosystems, due to high costs and logistical difficulties.

Keywords: intertidal, rock pools, complex network, food-web properties

2.1. Introduction

Comparative analysis of food webs from different habitats has revealed generalities in the subjacent network structure of trophic interactions. Estuarine, marine, stream, lake, and terrestrial ecosystems all seem to share similar general properties of complex food web network structure (Williams & Martinez, 2000; Camacho et al., 2002a; Dunne et al., 2004; Bascompte, 2009; Vinagre & Costa, 2014).

Initially, food web networks from marine ecosystems were thought to be different from those of other ecosystems (Cohen, 1994; Link, 2002), in that they presented higher average links per species, chain lengths and connectivity than non-marine ecosystems. Yet, Dunne et al. (2004) showed that those differences were due to different scales used in the analyses. Food web network properties are scale-dependent, changing as diversity and complexity change (Martinez, 1993, 1994) and thus direct comparisons can be misleading. In fact, Dunne et al. (2004) demonstrated that marine food webs are not different from nonmarine food webs, by comparing their fit to the theoretical niche food web model (Williams & Martinez, 2000). The niche model incorporates scale-dependence, hence allowing the comparison of food webs with different diversity and complexity.

Intertidal rocky shores are among the most intensely studied ecosystems in the world. They provide a natural laboratory where abiotic stress, biotic interactions and biological patterns can be easily examined (Thompson, 1980; Underwood, 1980; Menge et al., 1995; Hawkins et al., 2008). However, intertidal rock pools have received much less attention than the surrounding emergent intertidal bedrock, and thus much less is known about their community dynamics (Metaxas & Scheibling, 1993; Martins et al., 2007; Firth et al., 2014). This is mainly due to their high structural variability, which makes proper replication of sampling units very challenging (Metaxas & Scheibling, 1993; Underwood & Skilleter, 1996).

Intertidal rock pools are isolated mesocosms of permanently immersed habitat, surrounded by intermittently emerged rock surfaces. Environmental conditions in these pools are much less harsh than in the surrounding environment (e.g. high temperature amplitudes, desiccation stress). They allow many organisms to extend their upper vertical limits (Araújo et al., 2006; Firth & Crowe, 2008, 2010; Firth et al., 2013; Vinagre et al., 2015a), provide refuge (Vinagre et al., 2015a; Underwood & Jernakoff, 1984; Fairweather, 1988), feeding habitats (Wai & Williams, 2006; Dias et al., 2015) and nursery grounds (Dias et al., 2015; Bennett, 1987; Delany et al., 1998) for many marine species.

It is also generally acknowledged that the use of intertidal rock pools during early ontogeny (e.g. fish, shrimp) is likely to enhance growth, fitness and the survival chances of the individuals that use them (Mahon & Mahon, 1994; Macpherson, 1998; Strydom, 2008).

Intertidal rock pools are ubiquitous features of rocky shores in many parts of the world and can harbour rich biodiversity (Firth et al., 2014). Studies have been carried out focusing on their community structure (Menge et al., 1995; Martins et al., 2007; Underwood & Skilleter, 1996), and the roles of herbivory on community structure (Lubchenco, 1982, 1983; van Tamelen, 1996) competition (van Tamelen, 1996; Chapman & Johnson, 1990) predation (Lubchenco et al., 1984; Lubchenco, 1986; Menge et al., 1985) and recruitment (Chapman & Johnson, 1990). However, to the best of our knowledge, the network structure of food webs that occurs in intertidal rock pools remains unknown. The issue of whether complex food web networks can develop in such small and abiotically variable environments is yet to be uncovered. Given their accessibility and easy manipulation, these natural mesocosms could be useful models for the understanding of universal processes that regulate the complex organization of food webs, which are harder or impossible to investigate in open ecosystems.

The aim of the present study is to analyse, for the first time, the complex network structure of food webs occurring in intertidal rock pools and compare it to the ones of other habitats, by estimating their network properties and fit to the theoretical niche food web model (the network from each pool was compared to 1000 automatically generated food web networks). For this purpose, a significant number (n = 116) of intertidal rock pools were investigated in different biogeographic regions of the world, to encompass a wide range of potential variability, and compared to 46 other previously published food webs, from estuarine, marine, stream, lake, and terrestrial ecosystems. By doing this, we aim to investigate the potential use of rock pools as proxies of larger ecosystems for food web networks research.

2.2. Materials and methods

2.2.1. Sampling

The authors declare that the sampling followed the Portuguese, Brazilian, UK and Canadian legislation. Ethics committees in Portugal and Brazil specifically authorized this work. Authorization document 0421/000/000/2013 from the Portuguese authorities (DGAV) and 13.1.981.53.7 from the Brazilian authorities (CEUA, USP - Ribeirão Preto). The scientific permit delivered by Fisheries and Oceans Canada to Université du Québec à Rimouski, number

100003461, was used in Canada. No specific permissions were required for sampling in the field sites in the UK. The field work did not involve endangered or protected species in any of the areas.

Sampling took place always in summer (2013-2015), during spring tides. This time of the year was chosen, to ensure comparability since it is when biodiversity and species abundance is highest in the intertidal rock pools, compared to other seasons (personal observation). Six ecoregions were sampled: Gulf of Saint Lawrence-Canada, Celtic Sea-United Kingdom, South European Atlantic shelf-Portugal, Madeira Island-Portugal, Northeastern Brazil-Brazil, Southeastern Brazil-Brazil (Fig. 2.1).

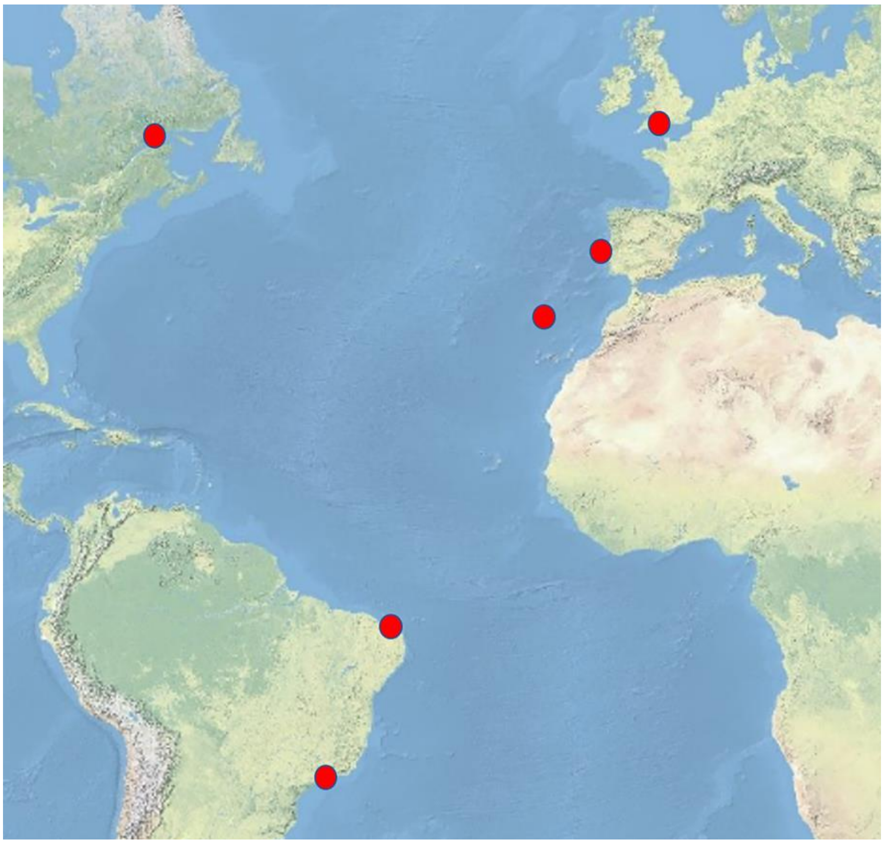

Figure 2.1. Location of the sampling sites. Red dots mark the location of the sampling sites.

Two sites were chosen in Canada (Gulf of St. Lawrence, site A-Pointe-au-Père- 48º29'33.0"N 68º29'33.0"W, site B-Sainte-Flavie- 48º36'43.0"N 68º 13'44.3"W), United Kingdom (South coast, site A-Mount Baten- 50º21'24"N 4º07'43"W, site B-Wembury- 50º19'00"N 4º04'57"W), Portugal-west coast (Portugal mainland, site A-Cabo Raso- 38º42'38.2"N 9º29'09"W and site B-Raio Verde- 39º17'11.4"N 9º20'23"W), Portugal- Madeira (Madeira Island, northeast Atlantic, site A-Caniço- 32º38'44.4"N 16º49'26.5"W, site B-Porto da Cruz- 32º46'32.6"N 16º49'33.5"W), Brazil-São Paulo (southeast coast, site A-São Sebastião-

23º49'26"S 45º25'38"W and site B-Ubatuba- 23º28'01"S 45º03'36"W) and Brazil- Ceará (Northeast coast, site A-Flecheiras- 3º13'04"N 39º15'29"W and site B-Guajirú- 3º 14'14"N 39º13'44"W). Sites A and B distanced between 6 and 60 km from each other. In these two sites, 2 to 4 beaches were targeted. All sampled intertidal rock pools were located in the lower intertidal and their size range (depth: 0.05 m - 0.80 m; surface area: 0.15 m^2 - 33.00 m^2, as estimated from scaled digital photographs using the software ImageJ) ensured a minimum patch size for the development of benthic assemblages, while still allowing a complete record of all macro-organisms found in each pool. In total, 28 pools were sampled in Canada, 8 in the UK, 32 in Portugal-west coast, 14 in Portugal-Madeira, 18 in Brazil-São Paulo (Brazil-SP) and 16 in Brazil-Ceará (Brazil-CE) (see Table SM2.1 in annex 2 in the annexes section, for the main pool characteristics).

Pool height (elevation-vertical distance from the mean sea level) and distance from the coastline was also registered. Substrate cover was registered, as well as water temperature (±0.1ºC) and salinity (±1‰). One bottom sediment sample of 50 ml was taken from pools with an area ≤ 0.5 m^2, two samples from pools with an area > 0.5 m^2 and ≤ 2 m^2, and three samples from pools with an area > 2 m^2, whenever the pool presented sediment at the bottom. Three quadrats of 5 cm^2 of rock pool surface were scraped. Sediment and scraping samples were preserved in alcohol 70º, with Bengal rose, and taken to the laboratory, where all organisms were identified with a stereomicroscope. Fish, shrimp and crabs were collected with hand-nets. Macroalgae, sponges, cnidarians, polychaetes, molluscs and echinoderms were collected by hand. All macro-organisms present in the pools were identified *in situ*, but samples were taken to the laboratory whenever there were taxonomical issues, requiring more detailed observation. In the latter case, marine organisms were identified with the aid of a stereomicroscope, and when necessary by consulting identification keys and taxonomic experts. Microscopic organisms were not included in the food webs, with the exception of zooplankton and phytoplankton that were included as a group due to low resolution of their predators' diet. Highly resolved food webs, depicting who eats whom, were compiled for each pool, based on published information on each species diet (see Text SM2.1, in annex 1 in the annexes section).

2.2.2. Network structure of food webs

The networks analysed were trophic species versions of the food webs. Trophic species are taxa that have the same set of prey and predators (Briand & Cohen, 1984). Using trophic species is a convention in structural network studies of food webs, in order to reduce methodological biases of uneven resolution among food webs (Williams & Martinez, 2000; Briand & Cohen, 1984). This food web networks consist of nodes connected by unweighted, directed links that represent prey-predator relations. For each food web, 11 basic properties of trophic species food webs were calculated (Table 2.1). A measure of biodiversity was included: number of trophic species (S). Two standard measures of food-web trophic interaction richness are reported: links per species (L/S), which indicates the mean number of links per node; and connectance (C), where $C = L/S^2$. Six properties yielded percentages of types of species in a food web: top (T) (taxa that lack any predators or parasites), intermediate (I), and basal species (B) (taxa that lack any prey items); cannibals (Can); omnivores (Omn) (taxa with food chains of different lengths, where a food chain is a linked path from a nonbasal to a basal species); and herbivores plus detritivores (H). Resource count and consumer count were also estimated for each trophic species. These are commonly estimated properties in food web network analyses (Williams & Martinez, 2000; Camacho et al., 2002a; Dunne et al., 2004).

Seven overall properties of trophic webs structure were also quantified (Table 2.1): mean shortweighted trophic level (TL), a trophic level measure which gives the most accurate estimate of trophic level based on binary link information (Williams & Martinez, 2004); mean number of links in every possible food chain, or sequence of links connecting top species to basal species (Chain); characteristic path length (Path), the mean shortest path length between species pairs; standard deviation of mean generality (GenSD) how many prey items a species has; vulnerability (VulSD), how many predators a species has; normalized standard deviation of links (LinkSD), which estimates links per taxon; and clustering coefficient (Clust), the mean fraction of species pairs connected to the same species that are connected to each other (Camacho et al., 2002b; Dunne et al., 2002; Montoya & Solé, 2002; Williams et al., 2002).

Table 2.1. Definition of the food web properties calculated.

Food web property	Definition of food web property
S	Number of trophic species
L/S	Links per species
C	Connectance, $C = L/S^2$
T	Top species (taxa that lack any predators or parasites)
I	Intermediate species
B	Basal species (taxa that lack any prey items)
Can	Cannibals
Omn	Omnivores (taxa with food chains of different lengths, where a food chain is a linked path from a non-basal to a basal species)
H	Herbivores plus detritivores
Resource count	Count of all species that serve as resources in the food web
Consumer count	Count of all species that serve as consumers in the food web
TL	Mean shortweighted trophic level
Chain	Mean number of links in every possible food chain or sequence of links connecting top species to basal species
Path	Mean shortest path length between species pairs
GenSD	Standard deviation of mean generality, how many prey items a species has
VulSD	Standard deviation of mean vulnerability, how many predators a species has
LinkSD	Normalized standard deviation of links, which estimates links per taxon
Clust	Clustering coefficient, the mean fraction of species pairs connected to the same species that are connected to each other

The software Network3D (Yoon et al., 2004; Williams, 2010) was used for all calculations. The ranges of the properties of the food webs examined were compared to those of highly resolved food webs published for other ecosystems (Table 2.2). The works selected for comparison with the results of the present work are recent works that apply a similar methodology to the one used in the field and lab in the present work and have been used for similar purposes in other recent works dealing with structural food web networks (e.g. Dunne et al., 2004; Mendonça & Vinagre, 2018). This study includes the same common trophic aggregations conducted in the other published works on food web networks used here for comparative purposes, which also rely on published works on diets. Generally, diet papers aggregate phytoplankton (because it is so difficult to analyse to the species level), zooplankton (for a similar reason); macroalgae (aggregated into large groups: red, brown and green macroalgae); oligochaeta and polychaeta (which are both often digested to a point where species' identification is not possible). The trophic aggregations are thus imposed by the establishment of feeding links based on published diet studies. This means that the level of resolution of the food webs analysed in the present work is as highly resolved as that previously published by other authors and that direct comparison of the networks is possible.

Linear regressions were calculated for the variation of food web properties with pool area, depth and height (for each location). Only significant correlations with a Pearson coefficient above 0.5 were considered. A significance level of 0.05 was used in all test procedures. All statistical analyses were carried out using the Statistica software (version 12.0, StatSoft Inc., USA).

Table 2.2. Ranges of commonly reported structural food-web properties for food webs from rock intertidal pools and a variety of other ecosystem types.

Ecosystem	N	S	C	L/S	T	I	B	Can	Omn	TL	Chain	Path	Source
Rock tide pools (all pools)	116	7–52	0.11–0.39	1.6–7.0	0–46	14–88	7–43	14–60	43–84	1.68–2.5	1.57–2.00	1.27–1.97	Present work
Rock tide pools 48°N, Gulf St. Lawrence–Canada	28	7–15	0.19–0.29	1.6–4.0	7–43	14–71	20–43	14–50	43–73	1.68–2.16	1.57–1.80	1.48–1.78	
Rock tide pools 50°N, UK	8	15–25	0.24–0.32	5.0–7.0	0–10	75–88	12–20	33–60	65–84	2.17–2.30	1.80–1.88	1.44–1.56	
Rock tide pools 38°N, Portugal-west coast	32	15–52	0.11–0.29	4.0–7.0	0–20	65–87	7–21	20–38	53–84	1.99–2.36	1.79–1.96	1.47–1.97	
Rock tide pools 32°N, Portugal-Madeira	14	11–24	0.20–0.39	3.0–5.0	0–10	62–79	16–27	31–58	62–79	2.05–2.35	1.73–1.84	1.27–1.72	
Rock tide pools 23°S, Brazil-SP	18	10–26	0.13–0.24	3.0–7.0	0–19	55–88	15–30	27–47	57–83	2.00–2.50	1.73–1.88	1.38–1.64	
Rock tide pools 3°S, Brazil-CE	16	11–26	0.19–0.33	2.0–3.0	8–46	27–77	12–27	11–33	64–84	1.90–2.41	1.7–2.0	1.59–1.93	
Seagrass beds	16	53–68	0.17–0.23	11.4–12.9	13–18	58–65	21–26	13–19	70–75	1.8–2.0	1.9–2.0	2.0–2.3	Coll et al., 2011
Marine	4	29–245	0.05–0.24	7.0–17.8	0–4	93–98	2–7	4–42	76–87	2.9–3.2	6.4–15.3	1.6–1.9	Dunne et al., 2004; Link, 2002; Optiz, 1996; Yodzis, 1998
Estuarine	12	48–117	0.03–0.14	2.0–10.1	7–52	31–86	4–20	1–24	53–84	2.4–2.9	4.0–6.6	2.0–2.7	Vinagre & Costa, 2014; Huxham et al., 1996; Lafferty et al., 2006; Hechinger et al., 2011; Dieter et al., 2007; Thieltges et al., 2011; Mouritsen et al., 2011
Lake/pond	5	25–172	0.12–0.32	4.3–25.1	0–9	66–92	4–32	12–32	38–60	2–2.7	4.0–10.7	1.3–1.9	Dunne et al., 2004; Warren, 1989; Havens, 1992; Martinez, 1991
Stream	5	31–109	0.07–0.13	3.7–7.6	6–25	22–86	7–56	1–2	6–10	1.5–3.4	3.1–3.2	2.3–2.3	Townsend et al., 1998; Romanuk et al., 2006
Terrestrial	4	29–155	0.03–0.31	1.6–9.0	0–31	56–90	13–18	0–66	21–76	2.4–3	3.2–8.4	1.4–3.7	Polis, 1991; Goldwasser & Roughgarden, 1991; Memmott et al., 2000; Waide & Reagan, 1996

S = number of trophic species, C = connectance, L/S = links per species, T = % top species, I = % intermediate species, B = % basal species, Can = % cannibalistic species, Omn = % omnivorous species, TL = mean trophic level, Chain = mean number of links in every possible food chain or sequence of links connecting top species to basal species, Path = characteristic path length (ranges that do not totally overlap with those of other non-marine ecosystems are presented in bold; ranges that do not totally overlap with those of other marine ecosystems are underlined).

2.2.3. The niche model

The capacity of the niche model (Williams & Martinez, 2000) in predicting food web properties was estimated for each intertidal rock pool food web. The niche model has 2 input parameters: the number of trophic species (S) and connectance (C) of the food web. The niche model assigns each species a randomly drawn `niche value' (n_i) from the interval (1, 0). Each species is then limited to consume all prey species within a range of values (r_i) whose randomly chosen centre (c_i) is less than the consumer's niche value. The niche model allows up to half a consumer's range to include species with higher niche values than the consumer, thus allowing looping (cycles of >1 length (e.g. A eats B, which eats A, or longer like A eats B, which eats C, which eats A)) and cannibalism (cycles of length 1 (A eats A)).

Additionally, the consumer must feed on all species that fall within its feeding range (r_i). For each food web, Monte Carlo simulations were used to generate 1000 niche model webs with the same S and C as the empirical web, allowing the estimation of a model mean and standard deviation for each of the network properties. If the normalized error (raw error divided by model SD) between the empirical property and the mean model value for that property falls with ± 1 model SD, the model is considered to have a good fit to the empirical data (Williams & Martinez, 2000). The software Network3D (Yoon et al., 2004; Williams, 2010) was used for all previous calculations. The percentage of niche model errors (taking into account all food web network properties) was estimated for each pool. Then the mean percentage of niche model errors was estimated for each location. A mean percentage of niche model errors <30% was considered a good fit (Dunne et al., 2004). Differences in the percentage of niche model errors among locations were analysed using a 1-way ANOVA, followed by Tukey post-hoc tests. The ANOVA assumptions were previously investigated. Normality was investigated through the Shapiro-Wilk's test and homoscedasticity through a Levene's test. A significance level of 0.05 was considered in all test procedures.

2.3. Results

The data assembled for the intertidal rock pool food webs resulted in lists of 11 to 68 taxa per pool. These taxa corresponded to lists of 7 to 52 trophic species per pool. Some biological compartments needed to be aggregated due to low definition of predator's diet, which impeded the construction of prey-predator links at the taxonomic species level. This was particularly evident for phytoplankton, zooplankton, oligochaeta and polychaeta.

The comparison of the range of food web network properties among intertidal rock pools and other ecosystems, reported in previous works (Table 2.2), showed that intertidal rock pools' properties are generally within the range estimated for other ecosystems.

The number of food web structural networks reported in the present work, n = 116, is remarkably higher than those previously reported for all other ecosystems combined, n = 46 (Table 2.2). The number of trophic species (S) observed in intertidal rock pools was considerably lower, 7-52, than that reported for all other ecosystems, 25-245, however such number of trophic species refers to much smaller areas (Table 2.2). Connectance in some intertidal rock pools was higher than that reported for other ecosystems (Table 2.2). Links per species (L/S) was lower, 1.6-7.0, than previously observed for marine systems, 7.0-17.8, but within the range reported for most non-marine ecosystems, 2.0-25.1. The percentage of intermediate species (I) showed lower bottom values, 14%, when compared to all other systems, 22-98%, due to the very low values reported for some pools in Canada. The highest values found for the percentage of basal species (B) and cannibal species (Can), 43% and 60%, respectively, were higher than those found for other marine systems, 26% and 42% respectively, however B was within the previously reported range for non-marine ecosystems, 4-56%, unlike Can which was not, since the percentage of Can in non-marine systems ranges between 1 and 32% (Table 2.2, Fig. 2.2). The lowest values found for the percentage of omnivorous species (Omn), 43%, were lower than reported for other marine systems, 70%, but within the ranges for non-marine ecosystems, 6-84% (Table 2.2, Fig. 2.2). Mean trophic level (TL), mean number of links in every possible food chain (Chain) and mean shortest path length between species pairs (Path) were within the values reported for non-marine ecosystems, however their lower values were lower than those reported for other marine ecosystems (Table 2.2).

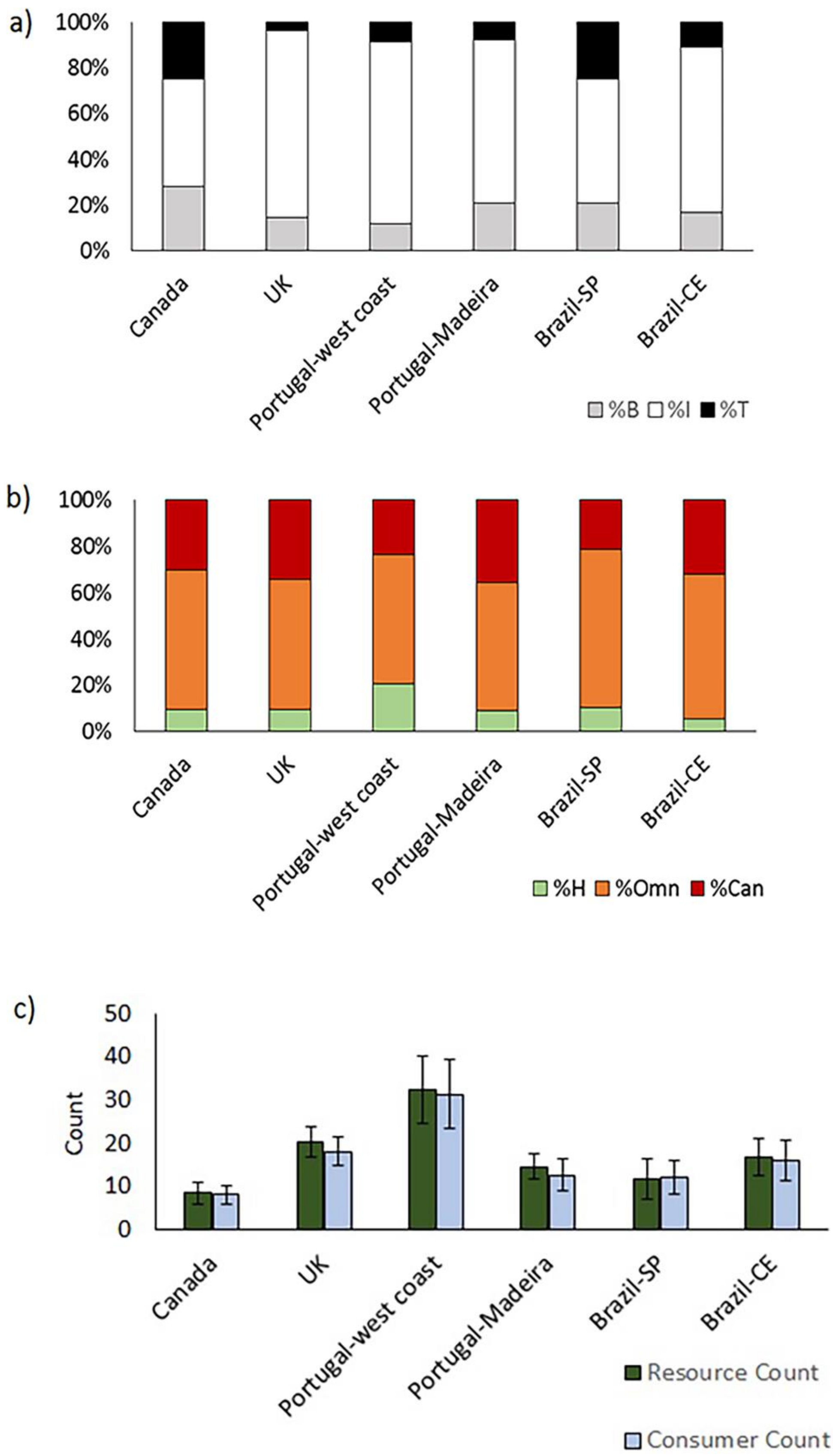

Figure 2.2. Variation in the basic properties of the food web networks of the rock intertidal pools. (a) percentage of top species (%T), percentage of intermediate species (%I) and percentage of basal species (%B); (b), percentage of herbivore species (%H), percentage of cannibal species (%Can) and percentage of omnivore species (%Omn) and (c) Resource and Consumer counts (mean values for all pools).

In addition, the taxa most frequently in the top 3 highest trophic level varied greatly among locations, however Polychaeta were among the top 3 in the UK, Brazil-SP and Brazil-CE, and the fish *Bathygobius soporator* in Brazil-SP and Brazil-CE (Table 2.3). The taxa that was most frequently in the top 3 highest trophic level was Oligochaeta in Canada, *Carcinus maenas* in the UK, Nematoda in Portugal-west coast, *Pachygrapsus transversus* in Portugal-Madeira, Polychaeta in Brazil-SP and in Brazil-CE (Table 2.3). The highest trophic level varied between 2.3 and 2.9 in Canada, between 2.8 and 2.9 in the UK, between 2.6 and 3.7 in Portugal-west coast, between 2.7 and 3.1 in Portugal-Madeira, between 2.6 and 3.6 in Brazil-SP and between 2.7 and 3.2 in Brazil-CE.

The taxa most frequently in the top 3 of highest connectivity were “detritus”, albeit not a taxon, this node was included in the webs and was always among the ones with highest connectivity, in all locations (Table 2.3). Zooplankton was also in this group in Canada, Portugal-Madeira, Brazil-SP and Brazil-CE (Table 2.3), and Polychaeta in Canada, the UK, Brazil-SP and Brazil-CE (Table 2.3). The highest values of connectivity varied between 1.5 and 2.0 in Canada, between 1.6 and 2.2 in the UK, between 1.1 and 3.9 in Portugal-west coast, between 1.4 and 2.1 in Portugal-Madeira, between 1.8 and 2.8 in Brazil-SP and between 1.7 and 2.5 in Brazil-CE.

Table 2.3. Taxa most frequently in the top 3 of highest trophic level* and connectivity in the food webs analyzed.

	Taxa most frequently in the top 3 highest trophic level*	Number of webs where the taxa were in the top 3 highest trophic level*	Taxa most frequently in the top 3 highest connectivity	Number of webs where the taxa were in the top 3 highest connectivity	Total webs analysed
Canada					28
	Oligochaeta	17	Zooplankton	28	
	Gammarus oceanicus (Amphipoda)	13	Detritus	27	
	Zooplankton	13	Polychaeta	10	
UK					8
	Carcinus maenas (Crab)	5	Polychaeta	8	
	Palaemon serratus (Shrimp)	4	Detritus	6	
	Polychaeta	3	*Carcinus maenas* (Crab)	6	
Portugal-west coast					32
	Nematoda	20	Detritus	32	
	Nassaridae (Snail)	16	*Lypophrys pholis* (Fish)	25	
	Anemonia sulcate (Anemone)	16	*Anemonia sulcata* (Anemone)	23	
Portugal-Madeira					14
	Pachygrapsus transversus (Crab)	7	Detritus	11	
	Palaemon elegans (Shrimp)	5	Zooplankton	10	
	Lypophrys pholis (Fish)	5	*Pachygrapsus transversus* (Crab)	4	
Brazil-SP					18
	Polychaeta	12	Detritus	18	
	Stramonita haemastoma (Snail)	9	Zooplankton	18	
	Bathygobius soporator (Fish)	4	Polychaeta	7	
Brazil-CE					16
	Polychaeta	10	Polychaeta	16	
	Bathygobius soporator (Fish)	8	Zooplankton	10	
	Pagurus sp. (Crab)	7	Detritus	9	

* shortweighted

No significant correlations were found for any of the food web properties and pool depth, area or height for the pools in Canada, Portugal-west coast, Portugal-Madeira and Brazil-CE. An important number of correlations ($r^2>0.5$; $p<0.05$) were found for the pools in the UK, for area and L/S ($r^2 = 0.9$), area and TL ($r^2 = 0.8$), area and I ($r^2 = 0.7$), area and resource count ($r^2 = 0.7$), area and S ($r^2 = 0.6$) and area and Omn ($r^2 = 0.5$). In Brazil-SP a correlation between area and L/S ($r^2 = 0.5$) was found.

The percentage of niche model errors ranged between 12% (Portugal-Madeira) and 27% (Portugal-west coast) (Fig. 2.3). The value was significantly higher for the intertidal rock pools surveyed in Portugal-west coast, in comparison to all other sites, with the exception of Brazil-SP ($p<0.001$; Fig. 2.3).

The food webs produced by Network3D (Yoon et al., 2004; Williams, 2010) allowed the visual observation of the complexity of the food web networks analysed. Examples of food web networks that depict various levels of biodiversity were selected and shown in Fig. 2.4, where the increasing complexity with increasing S is clear and easy to understand even by a non-specialized audience.

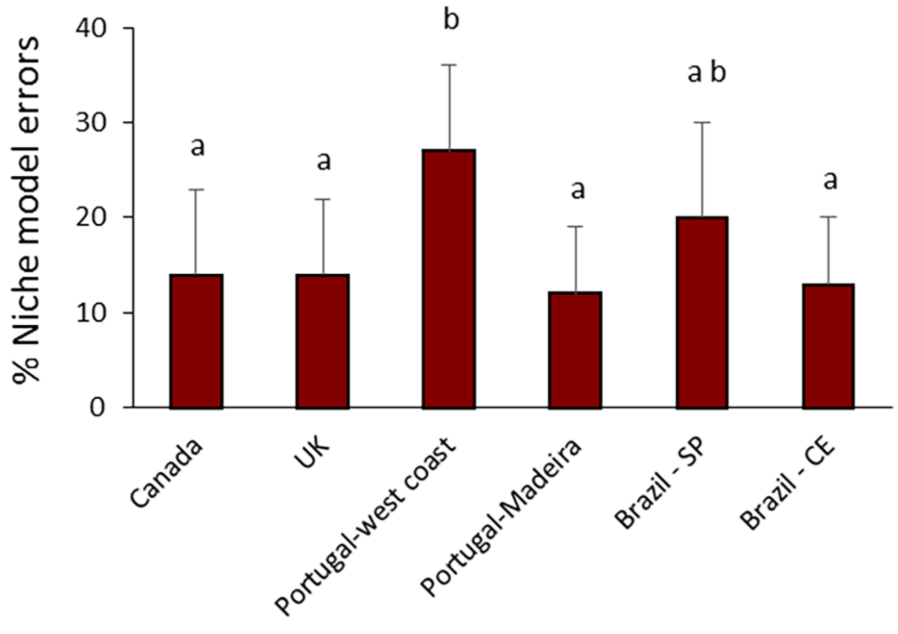

Figure 2.3. Percentage of niche model errors for 18 network structure properties (defined in Table 3) that are greater than |1|.

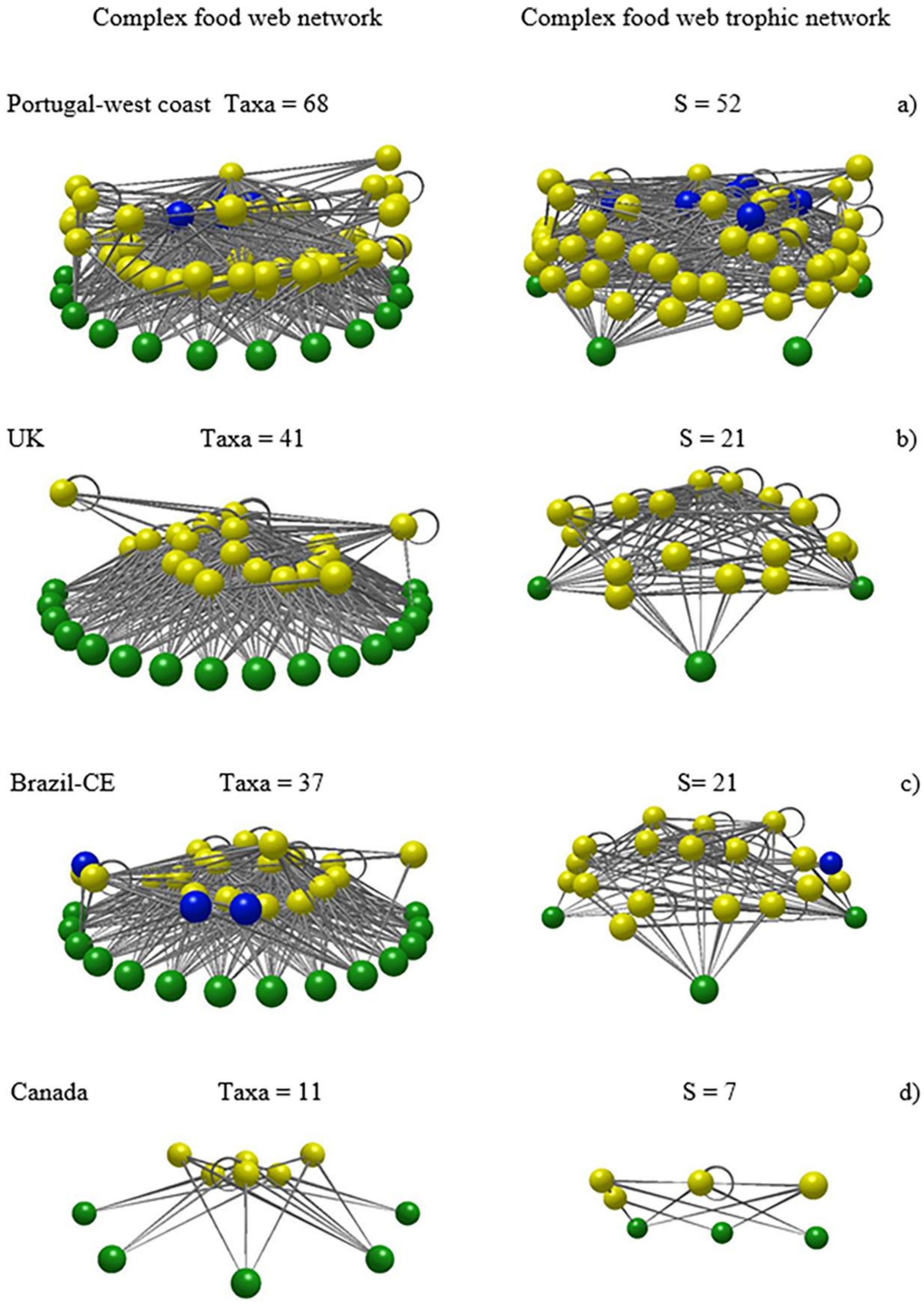

Figure 2.4. Network3D images of food web networks of selected rock intertidal pools. a-food web with the highest S, b and c-food webs with average S, d-food web in the lowest S. Green nodes = basal taxa; yellow nodes = invertebrates; blue nodes = vertebrates). On the left complex food web networks are depicted, on the right are the trophic species versions of the same food webs. Trophic species are groups of taxa whose members share the same set of predators and prey and are thus aggregated in single nodes.

2.4. Discussion

The clearest and most important conclusion of this work is that the food webs that occur in intertidal rock pools, albeit occupying very small areas, share the fundamental organizational structure of the food webs established over much larger open areas, such as marine areas, estuaries, rivers and terrestrial ecosystems, and thus can be useful for food web networks research.

Nevertheless, there were some differences in the ranges of properties found for intertidal rock pools when compared to other ecosystems. Connectance is usually highest in food webs with a large proportion of intermediate and omnivorous species, like marine food webs (Dunne et al., 2004). Intertidal rock pools had high connectance but a relatively low proportion of intermediate and omnivorous species. This high connectance was, in the case of intertidal rock pools, related to a high proportion of top, basal and cannibalistic taxa. This difference may reside in the role that intertidal rock pools play as refuge and feeding areas for early stages of predatory species, mostly fish, crabs and shrimp (Vinagre et al., 2015a; Dias et al., 2015; Dias et al., 2016). Such early stages, despite their small size, are often already predators of smaller animals that also find refuge and food in intertidal rock pools. The high proportion of cannibalism is also explained by the occurrence of such early stages, given that often the same species larvae and various juvenile stages will find refuge in the same pool, and the larger individuals cannibalize the smaller conspecifics (Vinagre et al., 2015a; Dias et al., 2015; Dias et al., 2016).

Dunne et al. (2004) hypothesized that the high proportion of intermediate, omnivorous and cannibal species found in marine food webs, when compared to non-marine food webs, could be related to: i) a resolution bias in marine datasets, that often present higher resolution for omnivorous commercial fish; ii) to a tendency to overlook cannibalistic relations in non-marine datasets or to iii) fundamental differences between the marine *versus* terrestrial food webs (e.g. widespread generality in marine systems based on gape size and the non-selectivity of filter feeding). These three hypotheses are discussed next.

Because intertidal species are well studied in the locations where the present study was conducted, some of the resolution biases that could be a problem in other marine studies were avoided, and that could be one of the reasons behind the lower proportion of intermediate and omnivorous species in intertidal rock pool food webs. The second hypothesis put forward by Dunne et al. (2004) that states that non-marine datasets overlook cannibalistic relations and have, therefore, a lower proportion of cannibalism could still hold true, although in the case of intertidal rock pools it can be argued the different sized life stages of predatory fish, crabs and shrimp find themselves together in a very small area, making cannibalism more unavoidable due to the impossibility of smaller life stages to escape. Pools are environments particularly prone to cannibalism (Whoriskey & FitzGerald, 1985; Ryer et al., 1997; FitzGerald et al., 1992; Parker et al., 2012) and the high proportion of cannibalism registered in the present study is probably an important particular characteristic of these food webs, as can be seen in the present study in the comparison between rock pools and large marine ecosystems (Table 2.2).

The results found for intertidal rock pools, albeit with some differences from other marine systems, also seem to support the third hypothesis put forward by Dunne et al. (2004), and previously proposed by Cohen et al. (1993), that marine systems have particular fundamental differences from non-marine systems, like feeding based on gape size and non-selective filter feeding by many primary and secondary consumers. The feeding based on gape size is the mechanism subjacent to the high proportion of cannibalism observed in intertidal rock pools (Dias et al., 2015). This is well known for fish, prone to eat any conspecific given that its size fits its mouth opening (Dias et al., 2015).

The low proportion of basal species in marine food webs, found by Dunne et al. (2004), was considered a clear artifact of low resolution of basal taxa and of the consumer links directed towards them. Dunne et al. (2004) concluded that an improvement in resolution at the basal level would mitigate, but not erase, the high levels of intermediate, omnivorous and cannibal species in marine food webs. In the present study, due to a good knowledge and abundant literature on intertidal macroalgae it was possible to eliminate this artifact. This resulted in a proportion of basal species higher that previously reported for other marine systems, but which in fact should be closer to the real values for marine systems in general, once higher resolution of basal species is achieved for other marine environments as well. Given that the proportion of intermediate and omnivorous species was lower for intertidal rock pools than previously reported for marine systems, it can be concluded that the present study supports the hypothesis of Dunne et al. (2004), apart from cannibalism, which remained high despite the better resolution at the basal level.

The highest trophic level found for Portuguese intertidal rock pools, between 2.6 and 3.7, confirms a previous isotopic study conducted in the same area over the intertidal platform, including intertidal rock pools, which placed the highest TL of that food web at 3.3 (Vinagre et al., 2015b). The species that occupied the highest trophic level varied widely among pools and locations, encompassing oligochaeta, polychaeta, anemones, amphipods, gastropods, crustaceans and fish. This probably reflects not only the environmental characteristics of each location, but also individual pool characteristics, such as depth, available prey and algal cover.

The taxon most frequently in the top 3 of highest connectivity, over all locations, was “detritus”. Albeit not technically a taxon it was considered a node in the food web. Its high connectivity confirms the previous findings by e.g. Vinagre et al. (2015b), Meziane & Tsuchiya (2000) and Bustamante & Branch (1996), which noted that intertidal food webs rely heavily on detritus.

Although an important number of correlations were found between pool area and some network properties in the UK, such size-related trends were not observed in the other locations, suggesting that limitations for the size and complexity of trophic networks may vary across ecoregions, and highlighting the need for replicate sampling at different spatial scales for a better appraisal of general patterns. The niche model predictive success was remarkably high (73-88%) for intertidal rock pools. This predictive success rate is similar to the 79% previously found for 7 non-marine food webs (Williams & Martinez, 2000) and the average 87% found for 3 marine food webs: the Benguela ecosystem off the coast of South Africa, a Caribbean coral reef ecosystem from the Puerto Rico-Virgin Islands shelf complex and a shelf ecosystem off the Northeast US (Dunne et al., 2004).

The overlap in properties' ranges between the rock pool food webs and previously published food webs (from a wide range of ecosystems) and their high fit to the niche model (among the highest ever published), lead to the conclusion that food web networks from rock pools have a great potential to be used as proxies of larger ecosystems for food web networks research. They are small and easy to sample, allowing greater replication and easy manipulation, two of the main challenges when dealing with large open systems. Although this approach would have some limitations, inherent to the use of one particular environment as proxy for vastly different environments and the uncertainty thereof, it would allow important advances resulting from the experimental manipulation of the web components and abiotic variables (e.g. algal coverage manipulation, predators' exclusion, temperature, salinity) over many replicate food webs.

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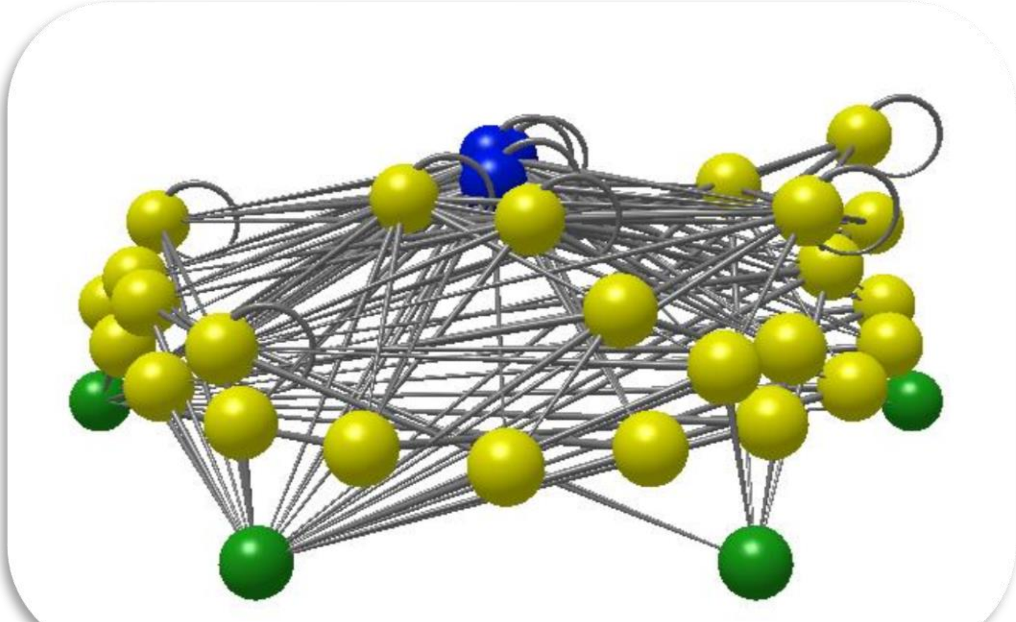
CHAPTER 3

Robustness of temperate and tropical tide pool food webs: comparing species trait-based sequential deletions



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Mendonça, V., Madeira, C., Dias, M., Silva, A.C.F., Flores, A.A.V, Vinagre, C. (submitted).

ABSTRACT

Loss of species can unleash a cascade of secondary extinctions that cause dramatic changes in the structure and dynamics of food webs. The consequences for the food web depend on the traits of the species that are lost so it is crucially important to identify species traits associated with secondary extinction risk. Another important issue is: where are the most vulnerable ecosystems? In this study, we aimed at comparing the robustness to species loss of temperate versus tropical ecosystems. For this, a total of 34 intertidal rock pools were analyzed from a temperate and a tropical region (17 pools in each). Robustness, a measure of network tolerance to species extinction, was assessed. Eighteen topological network properties were also compared, before and after the removals. Species loss was simulated *in silico* using sequential deletion protocols aimed at species that are: (1) most-connected; (2) least-connected; (3) most-abundant; (4) have the largest body mass and (5) the largest size. Tropical food webs revealed higher robustness than temperate food webs, however the tropical food webs' topology suffered more alterations after species loss. Both temperate and tropical food webs were less robust when the removal was directed at the most-connected species, confirming that highly-connected species are particularly important in food webs. The positive logarithmic relation previously found between robustness and connectance was only confirmed for temperate webs, highlighting the need for more tropical case-studies in general datasets.

Keywords: intertidal, rock pools, networks, secondary extinctions

3.1. Introduction

It is essential for Humanity that natural ecosystems maintain their healthy functioning. Unfortunately the biosphere is undergoing major changes nowadays, not only because of climate change but also due to overexploitation, pollution, invasion and degradation of habitats, which cause extinction of local populations and entire species worldwide (Hughes et al., 1997; Wilcove et al., 1998; Sala et al., 2000; Jackson et al., 2001; Baum et al., 2003; Lotze & Milewski, 2004; Myers & Worm, 2005; Lotze et al., 2006).

The role of ecologists is to try to understand how these extinctions affect the structure and functioning of ecosystems (Pimm et al., 1995; Loreau et al., 2002; Millennium Ecosystem Assessment, 2005). Several studies have suggested that analyzing the complex topological structure of food web networks is a useful step in the understanding of interrelations between several aspects of an ecosystem (e.g. species interactions, ecosystem structure and function) (e.g. Dunne et al., 2002b, 2016; Bascompte et al., 2005; Green et al., 2005).

Food webs networks describe the trophic relationships between species in an ecological system (Williams & Martinez, 2000; 2004; Dunne et al., 2002a; b; 2004; Montoya et al., 2006). In a topological representation of a food web network, nodes represent species and links represent who eats whom in the ecosystem (Williams & Martinez, 2000; Dunne et al 2002b; Proulx et al., 2005).

Mendonça et al. (2018), in a large-scale sampling effort covering 116 intertidal rock pools encompassing six ecoregions, revealed that intertidal rock pools' food webs were just as topologically complex as those of larger, open ecosystems, and that these microcosms' food webs fit the theoretical niche model put forward by Williams & Martinez (2000). This finding opened the way for the use of intertidal rock pools as models for the study of universal processes regulating the complex network organization of food webs (e.g. Brose et al., 2019).

The present work uses a subset of the data used by Mendonça et al. (2018) to investigate food web network robustness to species loss and, in particular, if there are differences between temperate and tropical food webs. The reason why only a subset of pools was selected for this study results from a standardization effort where physically similar pools (area, depth and elevation) where chosen in one temperate area (Portugal) and a tropical area (Brazil), so that the effect of those physical characteristics was minimized in the investigation of network robustness.

The comparison between temperate and tropical environments is important, given that conservation/management actions need to be prioritized in a world undergoing global change. The tropics are considered the cradle of biodiversity but are also home to vastly understudied ecosystems. The temperate zone is better studied but is where most of the human population is concentrated. Together, they make up most of the inhabited area of the globe. How robust are temperate and tropical ecosystems to species loss is a crucial question in the 21st century.

Robustness is the capacity to maintain functioning after a disturbance without fundamental changes (Dunne et al., 2002b; 2004; Memmott et al., 2004). Food web robustness has been studied by subjecting the webs to different species deletion sequences. Species are eliminated sequentially, each deletion is called primary extinction, and the extinctions resulting from such deletion are secondary extinctions (these occur when a consumer loses all its resources) (Dunne et al., 2002b).

A single real extinction event in response to one or more stresses, may precipitate cascades of further extinction acknowledged by the term “secondary extinction” (Pimm, 1980; Greenwood, 1987; Borrvall et al., 2000; Dunne et al., 2002b; Ebenman et al., 2004; Montoya et al., 2006; Dunne, 2009; Fowler, 2010). A robust system is a system where the number of secondary extinctions is low (Dunne et al., 2002b), that is, the higher the proportion of species that needs to be deleted, the more robust the web is.

The risk of cascading extinctions depends on the order of removal of the species, the number and function of the species removed, as well as on the trophic structure of the affected ecosystem (Solé & Montoya, 2001; Dunne et al., 2002b; Dunne & Williams, 2009; Staniczenko et al., 2010). Thus, robustness of a food web to species loss is nonrandom with respect to species identity but depend on the traits of the species that is lost from the community. Deletion sequences aimed at the most-connected species cause substantially more secondary extinctions than random removals (Solé & Montoya, 2001; Dunne et al., 2002b; 2004). Dunne et al. (2002b) found that robustness increases with food web connectance but appears to be independent of species richness and proportion of omnivory in the web. Dunne et al. (2004) concluded that marine food webs are fairly robust to species loss and attributed that to their relatively high connectance. Previous studies suffered from relatively low replication, because the assemblage of highly resolved food web networks is labor-intensive and time-consuming. Here, intertidal rock pools were used as microcosms that allow high replication of food web networks, to bring new insights into food web networks analysis.

This work compared the robustness (fraction of species that have to be removed for the loss of ≥50% of the total species) of temperate and tropical intertidal rock pool food webs, using sequential deletion protocols aimed at species that are: (1) most-connected; (2) least-connected; (3) most-abundant; (4) have the largest body mass and (5) the largest size. The effect of species removal was analyzed for 18 network properties.

3.2. Materials and methods

3.2.1. Study areas

A dataset composed of highly resolved food webs of 116 intertidal rock pools assembled by Mendonça et al. (2018) was used, of these, 34 pools were selected from a temperate (average SST = 17ºC) and a tropical (average SST = 25ºC) region, 17 tidal pools selected in Portugal-west coast (site A: Cabo Raso—38˚42’38.2"N 9˚29’09"W and site B: Raio Verde—39˚17’11.4"N 9˚20’23"W) and 17 tidal pools selected in Brazil-São Paulo (site A: São Sebastião—23˚49’26"S 45˚25’38"W and site B: Ubatuba—23˚28’01"S 45˚03’36"W).

All sampled intertidal rock pools selected were located in the lower intertidal and have similar size, depth range and elevation (surface area: 0.15 m^2 - 33.00 m^2; depth: 0.05 m - 0.80 m; elevation: 0.03 m - 2.2 m, for more details see Mendonça et al. (2018) and Fig. SM3.1 in annex 3). Data available at the iDiv Data Repository (https://doi.org/10.25829/iDiv.283-3-756).

3.2.2. Food-web topology

The networks assembled were based on trophic species. Trophic species are groups of taxa with 100% similarity in predator and prey (Briand & Cohen, 1984), aggregating nodes or taxa into trophic species (hereafter referred to as species) is a standard method used in structural food-web studies, in order to reduce methodological biases of uneven resolution of taxa within and among food webs (Briand & Cohen, 1984; Williams & Martinez, 2000).

For each food web 18 structural properties were calculated and compared before and after removals (Table 3.1). A measure of biodiversity was included: number of trophic species (S). Two standard measures of food-web trophic interaction richness are reported (Table 3.1): links per species (L/S) and connectance (C). Six properties give percentages of types of species in a food web (Table 3.1): top (T), intermediate (I), and basal species (B); cannibals (Can); omnivores (Omn) and herbivores plus detritivores (H). Resource count and consumer count were also estimated for each trophic species. Seven overall properties of trophic webs structure were also quantified (Table 3.1): mean short-weighted trophic level (TL); mean number of links connecting top species to basal species (Chain); characteristic path length (Path); standard deviation of mean generality (GenSD); vulnerability (VulSD); normalized standard deviation of links (LinkSD) and clustering coefficient (Clust).

3.2.3. Extinction analyses

The structural robustness (R_{50}) of food webs to species removal was calculated as the fraction of species that had to be removed to collapse the food webs to 50% or more (primary species removals and secondary extinctions) of their original size as defined by Dunne et al. (2002b). A secondary extinction occurs when a consumer species loses all of its prey items or when a cannibalistic species loses all of its prey items except itself. When the first primary extinction leads to the loss of 50% of the species there is minimum robustness (1/S) and when 50% of the species have been deleted and no secondary extinctions have occurred there is the maximum robustness (0.50) (Dunne et al., 2002b). The exact R_{50} is often overshot because species are discrete units. Lower values of R_{50} mean more secondary extinctions and, thus, lower robustness.

Species were removed, using sequential deletion protocols aimed at species that are: (1) most-connected; (2) least-connected; (3) most-abundant; (4) have the largest body mass and (5) the largest size. The structural robustness (R_{50}) was calculated as the average value for all webs analyzed in each criteria and region. The relation between robustness and connectance was investigated separately for temperate and tropical webs using a logarithmic regression.

3.2.4. Statistical analysis

To ensure that the temperate and tropical intertidal rock pools selected had similar area, depth and elevation t-tests were performed. For the calculations of network properties and extinction analysis the software Network3D was used (Yoon et al., 2004; Williams, 2010). The properties of the food webs before removals were compared to properties after removals and differences between tropical and temperate food webs were analysed using t-tests. Prior to these tests, normality and homoscedasticity were confirmed. A significance level of 0.05 was used in all test procedures. All statistical analyses were carried out using the Statistica software (version 12.0, StatSoft Inc., USA).

Table 3.1. Definition of the food web properties calculated.

Food-web properties		Description
Number of trophic species	S	Number of species in the food web after being converted into a trophic web
Links per species	L/S	Number of pred/prey links per species
Connectance	C	Proportion of actual trophic links to all possible links (L/S^2)
Top species	T	Species with prey and not predators or parasites
Intermediate species	I	Species with both predators and prey
Basal species	B	Species with predators and no prey
Herbivores plus detritivores	H	Species who prey on primary producers
Cannibals	Can	Species which prey on their own species
Omnivores	Omn	Species with food chains of different lengths, where a food chain is a linked path from a nonbasal to a basal species
Resource count		Count of all species that serve as resources in the food web
Consumer count		Count of all species that serve as consumers in the food web
Trophic level	TL	Trophic level averaged across taxa
Mean food chain length	Chain	Mean number of links in every possible food chain or sequence of links connecting top species to basal species
Mean shortest path lenght	Path	The mean shortest set of links between species pairs
Generality standard deviation	GenSD	Resources per taxon, how many prey items a species has
Vulnerability standard deviation	VulSD	Consumers per taxon, how many predators a species has
Normalized standard deviation of links	LinkSD	Links per taxon
Clustering coefficient	Clust	The mean shortest set of links between species pairs

3.3. Results

The temperate and tropical intertidal rock pools were similar in area (T = -0.11, p = 0.91), depth (T = -0.72, p = 0.47) and elevation (T = 1.20, p = 0.24). Temperate webs had more taxa (T = 8.61, p = 0.00) and links per species (T = 11.75, p = 0.00), than tropical webs. They also presented a higher proportion of intermediate (T = 6.84, p = 0.00), herbivorous (T = 9.04, p = 0.00) and cannibalistic species (T = 3.89, p = 0.00) than tropical webs. Tropical webs presented a higher proportion of top (T = -5.39, p = 0.00), basal (T = -7.07, p = 0.00) and omnivore species (T = -2.31, p = 0.03).

Tropical webs were more robust to removals than temperate webs based on the "most-connected" criterion (T = -3.31, p = 0.00, Fig. 3.1, Table 3.2). Tropical webs also presented lower values of extinctions per removal, than temperate webs (T = 2.68, p = 0.01, Table 3.2). However, the proportion of secondary extinctions that occurred were similar in the two regions (Table 3.2). For this criterion, after removals, there were significant differences in all tropical web properties, but only for 14 of 18 properties in temperate webs (Table 3.3, 3.4). Removals affected negatively intermediate species' proportion more than the other trophic groups, in the most-connect exercise, in the temperate webs (T = 1.34, p = 0.19, Table 3.4).

Temperate and tropical webs presented the same robustness to species removal according to the "least-connected" criterion (T = 1.34, p = 0.19, Figure 3.1, Table 3.2). However, tropical webs presented higher values of extinction per removal, than temperate webs (T = -2.08, p = 0.04, Table 3.2). The proportion of secondary extinctions was also higher in tropical webs (T = -2.17, p = 0.04, Table 3.2). For this criterion, after removals, there were significant differences in 13 of 18 properties in temperate webs and in 15 of 18 properties in tropical webs (Table 3.3, 3.4).

Tropical webs were more robust to removals based on the "most-abundant" criterion, than temperate webs (T = -2.30, p = 0.02, Fig. 3.1, Table 3.2). Tropical webs also presented lower values of extinctions per removal, than temperate webs (T = 2.51, p = 0.01, Table 3.2). However, the proportion of secondary extinctions were higher in temperate webs (T = 2.92, p = 0.00, Table 3.2). For this criterion, after removals, there were significant differences in 13 of 18 properties in both temperate and tropical webs (Table 3.3, 3.4).

The temperate and tropical webs presented the same robustness to species removal according to the "largest body mass" criterion (T = -1.48, p = 0.15, Table 3.2), and also did not present significant differences in values of extinction per removal and proportion of secondary extinctions (Table 3.2). For this criterion, after removal, there were differences in 9 of 18 properties in temperate webs and in 15 of 18 properties in tropical webs (Table 3.3, 3.4).

Tropical webs were more robust to removals based on the "largest size" criterion, than temperate webs ($T = -3.74$, $p = 0.001$, Table 3.2). However, temperate webs presented higher values of extinction per removal ($T = 3.57$, $p = 0.001$, Table 3.2) and higher proportion of secondary extinctions ($T = 3.62$, $p = 0.001$, Table 3.2), than tropical webs. For this criterion, after removals, there were significant differences in 15 of 18 properties in both temperate and tropical webs (Table 3.3, 3.4).

A positive logarithmic relation was found between robustness and connectance, for the deletion exercises based on most-connected, largest-size and largest body mass species, only for the temperate webs (Fig. 3.2). No significant relations were found for the tropical webs, on any of the deletion exercises (Fig. 3.2).

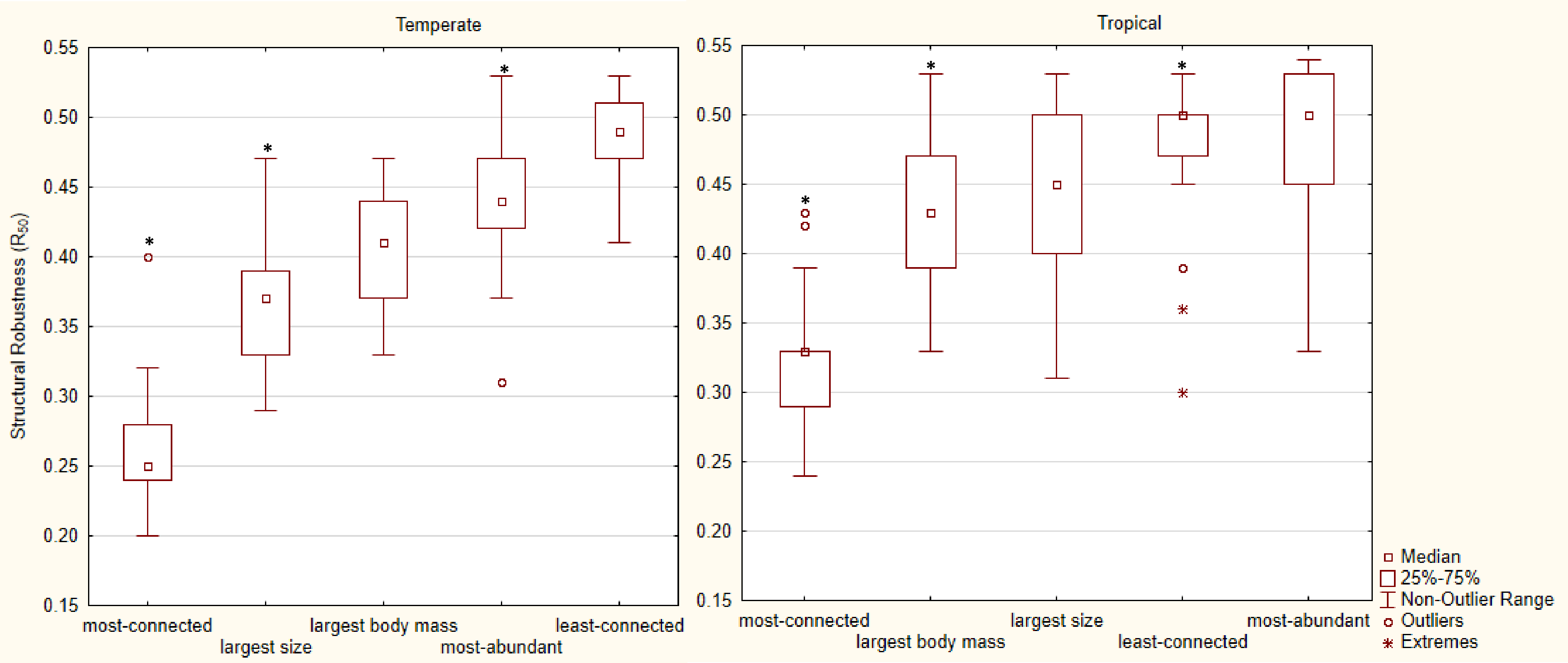

Fig. 3.1. The robustness to species loss. On the x-axis are the sequences, ordered by increasing robustness; on the y-axis is robustness measured as R_{50}.

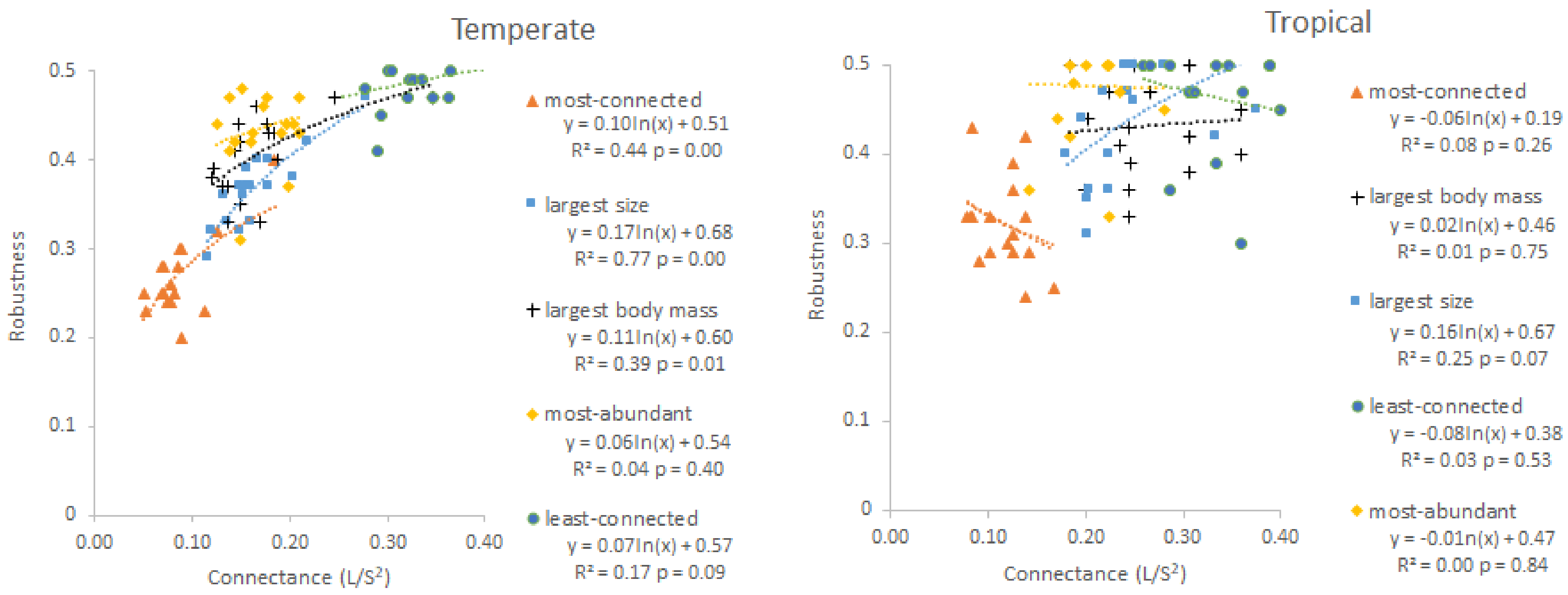

Fig. 3.2. Structural robustness as a function of connectance (L/S^2) in 17 food webs from temperate and 17 food webs from tropical region, for each criterion. Maximum robustness value = 0.50 (i.e. no secondary extinctions)

Table 3.2. Percentage of species removed and secondary extinctions in each criterion and region (Mean ± SD (min and max); red - significant differences between each region)

	Most-connected		Least-connected		Most-abundant		Largest body mass		Largest size	
	Temperate	Tropical	Temperate	Tropical	Temperate	Tropical	Temperate	Tropical	Temperate	Tropical
% species removed from initial S	26.79 ± 4.5 (20.0 - 40.0)	32.37 ± 5.30 (23.53 - 42.86)	48.81 ± 3.10 (40.38 - 53.33)	46.57 ± 6.19 (30.0 - 52.94)	43.43 ± 4.77 (30.77 - 53.33)	47.75 ± 6.10 (33.33 - 53.85)	40.56 ± 4.56 (32.69 - 47.37)	43.20 ± 5.79 (33.33 - 53.33)	36.66 ± 4.28 (29.84 - 46.67)	43.69 ± 6.45 (31.25 - 53.33)
Extinctions per removal	1.02 ± 0.30 (0.33 - 1.63)	0.73 ± 0.33 (0.20 - 1.75)	0.04 ± 0.06 (0.0 - 0.24)	0.15 ± 0.19 (0.0 - 0.67)	0.20 ± 0.15 (0.0 - 0.63)	0.08 ± 0.12 (0.0 - 0.40)	0.30 ± 0.13 (0.14 - 0.53)	0.24 ± 0.18 (0.0 - 0.6)	0.44 ± 0.15 (0.27 - 0.80)	0.23 ± 0.18 (0.0 - 0.60)
% secondary extintions from initial S	26.11 ± 4.71 (13.33 - 33.33)	22.44 ± 7.09 (8.33 - 41.18)	2.0 ± 2.53 (0.0 - 9.62)	5.74 ± 6.63 (0.0 - 20.0)	8.10 ± 5.01 (0.0 - 19.23)	3.30 ± 4.54 (0.0 - 14.29)	11.86 ± 3.85 (6.25 - 17.5)	9.32 ± 6.10 (0.0 - 20.0)	15.55 ± 3.58 (10.00 - 23.07)	9.27 ± 6.20 (0.0 - 18.75)
% S final over S initial	47.11 ± 2.28 (42.11 - 50.00)	45.18 ± 6.10 (30.77 - 50.0)	49.19 ± 1.03 (46.67 - 50.0)	47.69 ± 2.83 (40.0 - 50.0)	48.47 ± 2.37 (41.03 - 51.06)	48.94 ± 2.96 (45.45 - 58.33)	47.58 ± 3.52 (36.84 - 50.0)	47.48 ± 3.28 (40.0 - 50.0)	47.79 ± 3.15 (40.00 - 53.33)	47.04 ± 4.07 (36.36 - 50.0)

Table 3.3. Changes in properties after removals, where - means that the property decreases, + that the property increased, = the property did not change (red - when the change was significant, green - when the change was not significant).

	Most-connected			Least-connected			Most-abunbant			Largest body mass			Largest size		
	Temp	Both	Trop	Temp	Both	Trop	Temp	Both	Trop	Temp	Both	Trop	Temp	Both	Trop
Fundamental Properties															
S		-			-			-			-			-	
L/S		-		+		-		-			-			-	
C		-			+		+		+	+		+	+		+
Types of Taxa															
T		+		-		-	+		+		+		+		=
I		-		+		+		-			-			-	
B		+		+		-		+		+		+		+	
H	+		+		-		-		+	-		=	-		-
Omn		-			+		-		-	+		-	+		-
Can	-		-		+		-		-		+			+	
Network Structure															
GenSD	=		+		-		-		+	-		+	-		+
VulSD		-			-		+		-	+		-	+		-
LinkSD		-			-		-		-	-		-	-		=
TL		-		+		+		-		=		-		-	
Chain		-		-		+		-			-			-	
Path	+		-		-		-		+	-		-	+		-
Clust	-		-		+		-		+	+		+	-		+
ResourceCount		-			-			-			-			-	
ConsumerCount		-			-			-			-			-	

Table 3.4. Structural trophic web properties before and after removals (red indicates significant differences between each criterion and bold indicates significant differences between region).

	Temperate						Tropical					
		Most-connected	Least-connected	Most-abundant	Largest body mass	Largest size		Most-connected	Least-connected	Most-abundant	Largest body mass	Largest size
	before removal	**after removal**					**before removal**	**after removal**				
S	***35.88***	16.94	17.71	17.41	17.24	17.24	**14.71**	6.65	7.00	7.24	6.94	6.94
L/S	***5.32***	1.35	5.72	2.92	2.72	2.68	**2.45**	0.75	2.24	1.43	1.78	1.64
C	***0.16***	0.09	0.34	0.17	0.17	0.17	**0.17**	0.12	0.33	0.20	0.26	0.25
T	***0.09***	0.25	0.07	0.26	0.30	0.34	**0.26**	0.38	0.12	0.29	0.34	0.26
I	***0.80***	0.43	0.80	0.54	0.56	0.46	**0.52**	0.24	0.70	0.31	0.36	0.33
B	***0.12***	0.32	0.13	0.19	0.14	0.20	**0.21**	0.39	0.18	0.40	0.30	0.42
H	***0.25***	0.27	0.05	0.22	0.19	0.15	**0.10**	0.38	0.01	0.20	0.10	0.07
Omn	***0.64***	0.26	0.82	0.60	0.67	0.66	**0.69**	0.12	0.81	0.40	0.58	0.51
Can	***0.28***	0.26	0.45	0.22	0.31	0.31	**0.22**	0.13	0.46	0.19	0.25	0.28
GenSD	***1.12***	1.12	0.79	0.79	0.75	0.67	**0.66**	0.89	0.54	0.93	0.74	0.90
VulSD	***1.12***	0.89	0.67	1.23	1.36	1.31	**1.17**	0.86	0.73	0.92	0.95	0.85
LinkSD	***0.68***	0.60	0.30	0.55	0.66	0.59	**0.52**	0.30	0.24	0.52	0.47	0.52
TL	***2.23***	0.73	2.43	2.02	2.23	2.11	**2.11**	1.28	2.41	1.77	1.98	1.78
Chain	***1.89***	1.33	1.87	1.82	1.87	1.80	**1.82**	1.56	1.89	1.64	1.76	1.60
Path	***1.78***	2.50	1.37	1.75	1.72	1.79	**1.79**	1.44	1.42	1.82	1.53	1.64
Clust	***0.30***	0.27	0.38	0.24	0.32	0.27	**0.24**	0.12	0.36	0.28	0.33	0.35
ResourceCount	***32.76***	12.47	16.53	13.06	12.06	11.29	**11.06**	4.18	6.18	5.12	4.53	5.12
ConsumerCount	***32.00***	11.47	15.47	14.35	15.12	14.06	**11.65**	4.12	5.76	4.47	4.94	4.12

3.4. Discussion

This work showed, for the first time, that tropical food web networks are generally more robust to species loss than temperate food webs. The debate on the relative vulnerability of tropical *versus* temperate ecosystems has been centered, in recent years, mostly on global warming, and on how these two ecosystem types will respond to it, with most studies concluding that tropical ecosystems are more vulnerable and will probably lose more species in the future, than temperate ecosystems (e.g. Ghalambor et al., 2006; Tewksbury et al., 2008, Duarte et al., 2012, Vinagre et al., 2016, 2018, 2019). Such studies were based on experimental research on thermal tolerances, acclimation response and/or thermal safety margins of tropical and temperate species. The present study shows how important it is to move to the next scale of biological organization and investigate species interactions. The present robustness exercise indicates that, although tropical ecosystems will probably lose more species (in the context of climate warming), their food web networks seem to be more robust to such a loss than temperate webs. This brings a whole new perspective into this issue.

However, tropical webs generally suffered more alterations in its properties due to species loss than temperate webs. This could have negative or positive consequences, but it is important to consider since it adds complexity to an already complex topic. An ecosystem may be robust to species loss but if its food web network properties are greatly altered a variety of ecosystem functions may be compromised.

Various previous works reported that food web networks are particularly vulnerable to the loss of highly-connected species (e.g. Solé & Montoya, 2001; Dunne et al., 2002b; 2004; Memmot et al., 2004; Montoya et al., 2006; Curstdotter et al., 2011), which was confirmed in the present study, both for temperate and tropical webs. Tropical webs presented a robustness to the removal of species directed at the “most-connected” species of 32%, within the interval previously reported by Dunne et al. (2002a), which was between 30% and 60% for webs with a similar S, C, L/S and omnivory percentage (Chesapeake, Bridge Bay, Coachella and Skipwith, in Dunne et al. 2002a). Temperate webs, with a robustness of only 27%, revealed not only a lower level of robustness than tropical webs, but also lower than previously determined by Dunne et al. (2002a) for the four comparable webs mentioned above.

Dunne et al. (2004) reported higher robustness (on a most-connected exercise) for Caribbean reef webs and a NE US shelf web (>45%) than that found in the present study for both tropical and temperate webs. But for the Benguella marine web robustness was 30%, quite similar to that found in the present work.

Dunne et al. (2002b, 2004) found that robustness and connectance are logarithmically related (positively). The data for tropical and temperate webs falls out of this curve, having lower robustness than expected for their connectance level, similarly to the Benguela marine web analyzed by Dunne et al. (2004). Most importantly, this study showed, for the first time that, while a positive logarithmic relation exists for temperate webs, it does not for tropical webs. This shows that general patterns uncovered in temperate systems or with datasets where tropical systems are underrepresented should be taken with caution.

Albouy et al. (2019) found no detectable difference in robustness from the tropics to the poles. They did find differences in robustness between the open-sea and coastal areas, with coastal areas (0-200m depth) presenting considerably higher robustness. Albouy et al. (2019) is, however, not directly comparable to the present study because it is based exclusively on fish-to-fish interaction networks and the criteria used on the removals sequences is not equivalent to the ones used here.

The temperate food webs tested in the present study presented more than double the number of taxa and links per species than that of tropical webs, but similar connectance. The differences found in robustness confirm the findings by Dunne et al. (2002b), that concluded that robustness is independent of species richness. The higher number of taxa identified in the temperate webs may seem counterintuitive considering common assumptions on global biodiversity gradients. It is probably due to the fact that the Portuguese coast is a biodiversity rich region, where cold, temperate and subtropical species have overlapping ranges, which is common in mid-latitude regions (Cabral et al., 2001; Vinagre et al., 2011, 2019).

Temperate webs presented higher proportions of intermediate, herbivorous and cannibalistics species, removals affected intermediate species' proportion more than the other trophic groups, in the "most-connected" exercise. This way, we hypothesize that this high proportion of intermediate, highly connected species in the web, may result in less robust webs to the removal of highly connected species.

The higher robustness of tropical webs can be related with a number of aspects of the network topology prior to removals, such as highest proportion of top, basal and omnivorous species. The higher proportion of top species has already been related to higher robustness in previous studies (Carscallen et al., 2012). Also, basal species are of great importance in food webs, as any food web is supported by the presence of primary producers. Dunne et al. (2002b) showed that protecting basal species in removal exercises confers additional robustness to food webs at any particular connectance level. Omnivorous species are also stabilizing elements in food webs and reduce the likelihood of secondary extinctions, as previously reported (Fagan, 1997; McCann & Hastings, 1997; Borrvall et al., 2000; Bascompte et al., 2005).

Modeling of cascading extinctions in critical ecosystems has demonstrated that impacts of nonrandom species extinctions is markedly different from scenarios that assume that species extinction is random (Solé & Montoya, 2001; Dunne et al., 2002b). Although other studies have tested the effect of random species removal, this criterion was not used in the present study since the extinction of species in the marine environment does not appear to be random (Dunne et al., 2002b), particularly with respect to anthropogenic effects, which tend to impact high trophic levels, as is the case of fishing that tends to select top and larger species (Jackson et al., 2001; Coll et al., 2007). For this reason, the present study tested removals directed at the “largest body mass”, “largest size” and “most-abundant”, criteria that are relevant for fisheries but seldom tested in previous studies. It was concluded that robustness was considerably high (37-48%) for these criteria, both for temperate and tropical webs.

Curstdotter et al. (2011) compared robustness in purely topological and dynamical food web models (allometrically scaled, taking into account abundance and interaction strength), revealing that the topological approach overestimates robustness. In the topological approach, secondary extinctions occur only when a species loses all its prey, deeming all secondary extinctions the product of bottom-up cascades. Empirical observation as long showed that species loss can result in top-down cascades (e.g. Paine, 1966; Elmhagen & Rushton, 2007). There are numerous examples in nature of meso-predator control release caused by the loss of a top predator, resulting in the loss of lower level prey (e.g. Estes & Palmisano, 1974; Johnson et al., 2007; Elmhagen & Rushton, 2007). Similar effects can occur when top-down control ceases over strongly competitive species resulting in competitive exclusion (Paine, 1966; van Veen et al., 2005). Sahasrabudhe & Motter (2011) reported an example of such extensive secondary extinction cascades identified in dynamical food web models. This way, the present work, as all purely topological exercises, is a best-case scenario that only accounts for the minimum number of secondary extinctions, failing to identify top-down cascades.

Dynamic approaches, that model abundance and interaction strength, are thus more fitting for the study of robustness than purely topological approaches. However, in most ecosystems abundance and interaction strength are unknown for the vast majority of species. This is especially true for highly resolved food webs, as the ones used in the present work. This way, a topological approach is oftentimes the only possible approach.

At a time when some parts of the world (e.g. European Union) are promoting ecosystem-based approaches to achieve sustainability goals, such as maintenance of marine biodiversity, it is important to understand that such a commitment requires, first and foremost, a deeper understanding of biotic interactions. Topological studies, like the one presented here, are often the only option given data scarcity, hence important efforts should be done to deepen the existing knowledge on food webs all over the world. Nevertheless, the present study brings important new insights into food web structure and robustness for temperate and tropical ecosystems, being also one of the rare exercises in food web topology that uses an important number of replicate webs.

Future studies should use removal criteria based on realistic vulnerability rankings of species towards a stressor (e.g. high temperature, acidification, hypoxia, oil contamination) so that their impact on food webs can be simulated. For this to be possible, important efforts must be put in place to gather information for the most common species within a study-ecosystem. Such species' vulnerability to stressors must be tested so that vulnerability rankings are available for food web research. This will require a joint effort from field and experimental biologists, as well as from food web modelers.

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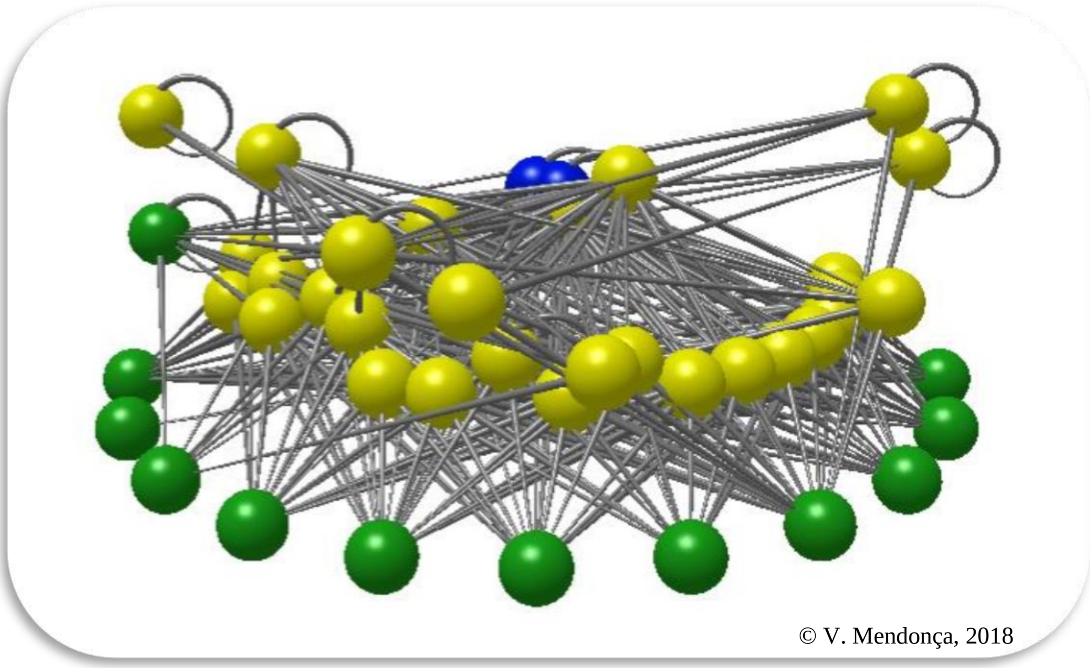
CHAPTER 4

Robustness of food web complex networks to heatwaves in tropical and temperate shallow waters



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Mendonça, V., Madeira, C., Dias, M., Silva, A., Flores, A.A.F., Vinagre, C. (submitted).

ABSTRACT

The structural robustness of food web networks to species loss has been mostly addressed in theoretical exercises based on unrealistic extinction sequences. It is thus important to test ecologically relevant extinction sequences, so that a forecast capacity on food web disturbances can be achieved. This work tests, for the first time, an extinction sequence based on species thermal vulnerability, a relevant criterion in a warming world. Prior to this work, a ranking of thermal vulnerability was experimentally established for the most common species occurring in the model-ecosystem tested: intertidal rock pools. This was done in temperate and in tropical pools (17 pools in each zone), so that the effect of heat waves in the temperate and in the tropical zone could be compared. The pools were surveyed so that their thermal conditions were known. Highly resolved food web networks were also available for each pool. Two removal exercises were conducted: 1) species were sequentially removed from the food webs according to their thermal vulnerability to determine food web network robustness (the fraction of species that need to be removed to result in the loss of ≥ 50% of the total species), and 2) species were sequentially removed from the food webs according to their thermal vulnerability but removal stopped when it reached a species with a critical thermal maximum (CTMax) higher than the maximum habitat temperature (MHT). The second exercise intends to mimic the effects of a heatwave in present thermal conditions. Temperate and tropical food web networks presented similar robustness, however the tropical food web networks presented alterations in more food web properties. The heatwave exercise showed that the tropical networks lose a much higher number of species and present much more alterations in network properties, because a much higher number of species had a CTMax below MHT and were thus removed. This work shows that although temperate and tropical webs present similar structural robustness to the removal of species based on thermal vulnerability, tropical food webs are much more likely to suffer network alterations because they encompass more vulnerable species.

Keywords: climate warming, global change, rocky shore, trophic ecology, tide pools

4.1. Introduction

Sweeping losses of biodiversity are occurring worldwide, caused by habitat destruction, alien species introduction, climate change, and pollution (Wilcove et al., 1998). The robustness of ecosystems to species losses is a central question in ecology, it is crucial to understand and predict how the structure of ecological networks influences extinction patterns (Loreau et al., 2001; Raffaelli, 2004; Worm et al., 2006), and understand how extinction affects the functioning of ecosystems (Pimm et al., 1995; Loreau et al., 2002; Millennium Ecosystem Assessment, 2005).

There is evidence that tropical organisms are more vulnerable to warming than their temperate counterparts (Tewksbury et al., 2008; Vinagre et al., 2016, 2018, 2019a). Although temperate latitudes are expected to warm faster than the tropics (IPCC, 2007, 2013), the species living in the tropical habitats exhibit lowest thermal plasticity (Chown, 2001; Overgaard et al., 2011; Gunderson & Stillman, 2015) and live closer to their upper thermal limits, so they have been considered more vulnerable to warming (e.g. Deutsch et al., 2008; Vinagre et al., 2016, 2018, 2019a).

However, we don’t know what this means for the food webs where they occur. Are tropical food webs more vulnerable to warming than temperate food webs? They are more likely to lose species, but we don’t know what that means for their network organization. At a time when warming threatens biodiversity, ecosystem function and human well-being at a global scale, it is crucial to prioritize environmental management through the identification of relative vulnerability of species, habitats and ecosystems (Williams et al., 2008).

However, working at the habitat or ecosystem scale presents complicated logistical challenges and high costs. It is crucial to find natural microcosms that can be used as proxies. Natural microcosms should be as complex and biologically realistic as larger natural systems so that they can be candidate model systems for ecology (Srivastava et al., 2004). In addition to being relatively simple to describe, their small size should enable high replication in experiments and their contained structure should allow a precise delineation and tractability of communities (Srivastava et al., 2004).

Intertidal rock pool environments are small environments, they have clear boundaries, sampling a high number is feasible and they present high biodiversity. Also, they are particularly vulnerable to warming due to their small volume (Hiatt & Strasburg, 1960; Little et al., 2009; Vinagre et al., 2016, 2018, 2019a). During heat waves they can warm to much higher temperatures than coastal waters (IPCC 2007, 2013; Robertson et al., 2013; Vinagre et al., 2016, 2018).

Recently, Mendonça et al. (2018) showed that intertidal rock pools present just as much food web network complexity as larger, open ecosystems, by analyzing the food web network topology of a high number of intertidal pools (n=116) from a vast array of latitudes and ecoregions of the world - Celtic Sea (United Kingdom, 50ºN), Gulf of Saint Lawrence (Canada, 48ºN), South European Atlantic shelf (Portugal, 38ºN), Madeira Island (Portugal, 32ºN), Northeastern Brazil (Brazil, 3ºS) and Southeastern Brazil (Brazil, 23ºS), concluding that these microcosms can be useful proxies for larger ecosystems.

To predict the response of a natural ecosystem to species loss it is necessary to know the structure of the system as well as which species are susceptible to extinction in the first place. Thus, in this work we analyze the structure of the systems recently defined by Mendonça et al 2018 and the species susceptible to extinction determined by Vinagre et al. (2018).

The aim of the present work was to compare the robustness of tropical and temperate food web networks to the sequential removal of species according to a ranking of thermal vulnerability previously established experimentally by Vinagre et al. (2018). Few studies have investigated the response of natural ecosystems to realistic extinction sequences (Srinivasan et al., 2007; de Visser et al., 2011) so this work is an important step forward.

Additionally, another removal exercise was carried out where species were sequentially removed from the food webs according to their thermal vulnerability, but removal stopped when a species with a critical thermal maximum (CTMax) higher than the maximum habitat temperature (MHT) was reached. CTMax and MHT were previously determined for these same pools by Vinagre et al. (2018) and Mendonça et al. (2018), respectively. This second exercise intends to mimic the effects of a heatwave, where MHT is reached, in the present thermal conditions of intertidal rock pools.

Highly resolved food webs were assembled for 34 intertidal rock pools from a tropical and a temperate region (a subset of the food webs analyzed by Mendonça et al. (2018)), in Brazil (23ºS) and Portugal (38ºN) and their structural robustness was estimated. The network robustness is a measure that assesses the tolerance capacity of a web to maintain its structure after species extinctions and is defined as the fraction of species that need to be removed to result in the loss of ≥ 50% of the total species (Dunne et al., 2002a, 2005; Memmott et al., 2004; Donohue et al., 2016).

4.2. Materials and methods

4.2.1. Field study

Mendonça et al. (2018) compiled highly resolved food webs for 116 intertidal rock pools from cold, temperate, subtropical and tropical regions. The network properties of these food webs were calculated and compared to that of estuaries, lakes, rivers and marine and terrestrial ecosystems, showing, that intertidal rock pools can be useful models for the understanding the complex network organization of food webs (Mendonça et al., 2018). Of the 116 intertidal rock pools assembled in Mendonça et al. (2018) 34 pools were selected from a temperate (average SST = 17ºC) and tropical (average SST = 25ºC) region, 17 in Portugal-west coast (Portugal mainland, site A–Cabo Raso—38˚42’38.2"N 9˚29’09"W and site B–Raio Verde—39˚17’11.4"N 9˚20’23"W) and 17 in Brazil-São Paulo (southeast coast, site A–São Sebastião—23˚49’26"S 45˚25’38"W and site B–Ubatuba—23˚28’01"S 45˚03’36"W). All sampled intertidal rock pools selected were located in the lower intertidal and have similar size and depth range (surface area: 0.15 m^2 - 33.00 m^2; depth: 0.05 m - 0.80 m), allowing a record of all macro-organisms in each pool and guaranteed the minimum size to allow the development of benthic assemblages. For the tide pools in the tropical site the maximum habitat temperature (MHT) was 41.5ºC and for the temperate site was 30.6ºC (for more details see Vinagre et al., 2018).

4.2.2. Experimental study

Vinagre et al. (2018) and Vinagre et al. (2019) tested the upper thermal limits (Critical Thermal Maxima, CTMax) and acclimation capacity of 88 coastal species (Gastropoda, Crustacea, Teleostei, Echinodermata and Cnidaria) that occur in the same tropical and temperate sites from this study. Upper thermal limits experimentally determined allowed the assemblage of rankings of thermal vulnerability, used for the sequential removal of species (Table 4.1, 4.2).

Table 4.1. Species removed the upper thermal limits, from CTMax lowest to highest.

Temperate	CTMax	Tropical	CTMax
Atherina sp.	27.27	*Cerithium atratum*	37.06
Ophiurus sp.	29.77	*Scartella cristata*	38.14
Nassarius reticulatus	30.24	*Microphyrys bicornutus*	38.48
Asterina gibbosa	31.18	*Palaemon northropi*	38.84
Lepadogaster lepadogaster	31.77	*Ischnochiton striolatus*	39.10
Lophozozymus incisus	32.07	*Tegula viridula*	39.35
Leptochiton algesirensis	32.33	*Pachygrapsus transversus*	39.80
Coryphoblennius galerita	32.55	*Strombus pugilis*	39.87
Pirimela denticulata	32.60	*Eryphia gonagra*	39.99
Necora puber	32.62	*Callinectes danae*	40.32
Lipophrys trigloides	32.97	*Eurypanopeus abbreviatus*	40.34
Marthasterias glacialis	33.01	*Bathygobius soporator*	40.56
Gibbula umbilicalis	33.45	*Odontesthes argentinensis*	40.82
Diplodus sargus	33.56	*Echinolittorina lineolata*	41.37
Lipophrys pholis	33.83	*Clibanarius antillensis*	41.43
Lepidochitona cinerea	34.20	*Lottia sobrugosa*	41.58
Palaemon serratus	34.63	*Morula nodulosa*	41.67
Acantochitona crinita	34.67	*Stramonita haemastoma*	41.90
Gobius paganellus	34.68		
Littorina saxatilis	34.91		
Palaemon elegans	35.03		
Phorcus lineatus	35.26		
Pachygrapsus marmoratus	35.28		
Actinia equina	35.67		
Carcinus maenas	36.00		
Eriphia verrucosa	37.10		
Patella depressa	37.45		
Patella vulgata	38.85		
Mytillus galloprovinciallis	40.74		

Table 4.2. Species removed with thermal limits below the maximum habitat temperature (MHT), from CTMax lowest to highest.

Temperate	CTMax	Tropical	CTMax
Atherina sp.	27.27	*Cerithium atratum*	37.06
Ophiurus sp.	29.77	*Scartella cristata*	38.14
Nassarius reticulatus	30.24	*Microphyrys bicornutus*	38.48
		Palaemon northropi	38.84
		Ischnochiton striolatus	39.10
		Tegula viridula	39.35
		Pachygrapsus transversus	39.80
		Strombus pugilis	39.87
		Eryphia gonagra	39.99
		Callinectes danae	40.32
		Eurypanopeus abbreviatus	40.34
		Bathygobius soporator	40.56
		Odontesthes argentinensis	40.82
		Nodillitorina lineolata	41.37
		Clibanarius antillensis	41.43

4.2.3. Sequential removal of species

For each food web, in a tropical and a temperate area, we simulated species loss by sequentially removing species selected by their thermal vulnerability. In the present work species were removed by 2 criteria: (1) the upper thermal limits, from CTMax lowest to highest (Table 1) and (2) species with thermal limits below the maximum habitat temperature (MHT), from CTMax lowest to highest (Table 4.2).

The impact of species loss is examined by the number of potential secondary extinctions. A secondary extinction occurs when a species loses all of its prey items. The basal species do not experience secondary extinctions only primary removals (Dunne et al., 2002a).

Robustness (R_{50}) of food webs to species loss was calculated as the fraction of species that are removed to lose (primary species removals plus secondary extinctions) $\geq$ 50% of the total species in the original web. When the first removal leads to the loss of 50% of the species, there is minimum robustness (1/S) and when no secondary extinctions follow primary extinction of 50% of the species there is maximum robustness (0.50) (Dunne et al., 2002a).

4.2.4. Statistical analysis

For the analysis of extinction sequences, robustness and food web properties the software Network3D was used (Williams, 2010). Eighteen basic network structure properties were calculated, for each food web (Table 4.3). Differences for each of the network property before and after sequential removals and differences between tropical and temperate networks were analysed using a one-way ANOVA, followed by Tukey post-hoc tests. Prior to these tests, normality and homoscedasticity were confirmed. A significance level of 0.05 was considered, in all test procedures.

Table 4.3. Definition of the food web properties calculated.

PROPERTY	DEFINITION OF FOOD WEB PROPERTY
S	Number of trophic species in the food web
L/S	Links per species
C	Connectance, $C = L/S^2$
T	Top species (taxa that lack any predators or parasites)
I	Intermediate species
B	Basal species (taxa that lack any prey items)
CAN	Cannibals
OMN	Omnivores (taxa with food chains of different lengths, where a food chain is a linked path from a non-basal to a basal species)
H	Herbivores plus detritivores
RESOURCE COUNT	Count of all species that serve as resources in the food web
CONSUMER COUNT	Count of all species that serve as consumers in the food web
TL	Mean shortweighted trophic level
CHAIN	Mean number of links in every possible food chain or sequence of links connecting top species to basal species
PATH	Mean shortest path length between species pairs
GENSD	Standard deviation of mean generality, how many prey items a species has
VULSD	Standard deviation of mean vulnerability, how many predators a species has
LINKSD	Normalized standard deviation of links, which estimates links per taxon
CLUST	Clustering coefficient, the mean fraction of species pairs connected to the same species that are connected to each other

4.3. Results

When species were systematically removed from the food webs, from lowest to highest CTMax, no significant difference in terms of robustness was detected between tropical and temperate networks, that is, the proportion of species removed was similar (Table 4.4). The proportion of secondary extinctions that resulted from the removals was also similar (Table 4.4).

Although the robustness of tropical and temperate food webs was similar, the tropical webs experienced significant alterations in more network properties (Table 4.5, 4.6). Of eighteen network properties calculated, after the sequential removals ten properties suffered significant differences in temperate food webs and fourteen in tropical food webs (Table 4.5, 4.6). In the fundamental properties of the food web network structure, in both regions, there was a significant decrease in the number of species (S) and the number of links (L/S), while the connectance (C) significantly increased in the tropical region (Table 5 and 6). There was a significant decrease of intermediate (I) and the significant increase of percentage of top (T) taxa in both regions. In the temperate region the herbivores plus detritivores (H) taxa significant decreased and in the tropical region there was a significant increase in the basal (B) taxa and a significant decrease in omnivores (Omn) taxa (Table 4.5 and 4.6). In the network structure of temperate region there was a decrease in the variability of number of resources per taxon (GenSD), in the variability of consumers per taxa (VulSD), in the variability of links per taxon (LinkSD) and the probability that two taxa linked to the same taxon are linked (Clust); in temperate region the number of resources per taxon (GenSD), links per taxon (LinkSD) and the probability that two taxa linked to the same taxon are linked (Clust) increased significantly and the trophic level (TL) and the mean number of links in every possible food chain (Chain) decreased significantly. Resource count and consumer count decreased significantly in both regions.

When species with thermal limits below the maximum habitat temperature were removed (following a thermal vulnerability sequence similar as in the previous exercise) the proportion of species removed was different between temperate and tropical food webs, with significantly more removals in the tropical webs (83% versus 10%). However, there were no significant differences in the proportion of secondary extinctions (Table 4.5).

More network properties changed in the tropical webs. In the temperate webs, not enough species were removed to create alterations in the properties of the trophic network (Table 4.5, 4.6). The opposite occurred in tropical food webs where ten properties suffered significant differences after removals. (Table 4.5, 4.6). In the fundamental properties of the tropical food web network structure there was a significant decrease in the number of species (S) and the number of links per species (L/S) (Table 4.5 and 4.6). In the types of taxa there was a significant decrease of intermediate (I) and omnivores (Omn) taxa and the significant increase of percentage of top (T) and basal (B) taxa (Table 4.5 and 4.6). In the network structure the trophic level (TL) and the mean number of links in every possible food chain (Chain) decrease significantly, and the variability of links per taxon (LinkSD) increased significantly. Resource count and consumer count decreased significantly (Table 4.5 and 4.6).

Table 4.4. Proportions obtained in temperate and tropical zones for the two criteria of removal, Mean ± SD (min and max), red indicates significant differences between each region.

	CTMax lowest to highest		CTMax below MHT	
	Temperate	**Tropical**	**Temperate**	**Tropical**
% species removed from initial S	29 ± 7.0 (15-47)	26 ± 7.0 (14-40)	4.0 ± 1.0 (3-7)	20 ± 8.0 (7-35)
Extinctions per removal	0.3 ± 0.2 (0-0.6)	0.3 ± 0.3 (0-1)	0.1 ± 0.4 (0-2)	0.1 ± 0.2 (0-0.5)
% secondary extinctions from initial S	8.0 ± 5.0 (0-19)	7.0 ± 6.0 (0-19)	0.7 ± 2.0 (0-10)	3.0 ± 5.0 (0-17)
% S final over S initial	62 ± 8.0 (50-80)	67 ± 9.0 (50-80)	95 ± 3.0 (85-97)	78 ± 11 (50-93)

Table 4.5. Significantly changes observed in the properties of the trophic network.

	CTMax lowest to highest		CTMax below MHT	
Properties	**Temperate**	**Tropical**	**Temperate**	**Tropical**
SpeciesCount (S)	↘	↘		↘
LinksPerSpecies (L/S)	↘	↘		↘
Connectance (C)		↗		↗
FracTop (T)	↗	↗		
FracIntermed (I)	↘	↘		↘
FracBasal (B)		↗		↗
FracHerbiv (H)	↘			
GenSD	↘			
VulSD	↘	↗		↗
LinkSD	↘	↗		
MeanSWTL (TL)		↘		↘
MeanShortChn (Chain)		↘		↘
FracOmniv (Omn)		↘		↘
FracCannibal (Can)				
CharPathLen (Path)				
MeanClusterCoeff (Clust)	↘	↗		
ResourceCount	↘	↘		↘
ConsumerCount	↘	↘		↘

Table 4.6. Trophic web properties, before and after the sequential removal of species according to the two removal criteria, in the temperate and tropical zone (red – indicates significant differences between before and after the sequential removal).

	CTMax lowest to highest						CTMax below MHT					
	Temperate			Tropical			Temperate			Tropical		
	before	after	%	before	after	%	before	after	%	before	after	%
SpeciesCount (S)	35.88	22.29	-37.87	14.71	9.76	-33.60	35.88	34.00	-5.25	14.71	11.35	-22.80
LinksPerSpecies (L/S)	5.32	3.57	-32.81	2.45	1.85	-24.52	5.32	5.17	-2.75	2.45	1.97	-19.49
Connectance (C)	0.16	0.17	6.39	0.17	0.20	15.56	0.16	0.16	2.75	0.17	0.18	6.40
FracTop (T)	0.09	0.15	79.72	0.26	0.37	42.88	0.09	0.09	1.38	0.26	0.38	47.29
FracIntermed (I)	0.80	0.69	-13.25	0.52	0.31	-41.50	0.80	0.79	-0.91	0.52	0.34	-35.12
FracBasal (B)	0.12	0.15	31.70	0.21	0.32	49.46	0.12	0.12	5.20	0.21	0.28	28.53
FracHerbiv (H)	0.25	0.21	-18.00	0.10	0.07	-32.44	0.25	0.26	4.11	0.10	0.08	-22.47
GenSD	1.12	0.94	-16.24	0.66	0.75	13.40	1.12	1.13	0.34	0.66	0.69	4.30
VulSD	1.12	1.18	5.80	1.17	1.17	-0.70	1.12	1.09	-2.73	1.17	1.24	5.88
LinkSD	0.68	0.60	-12.01	0.52	0.57	10.48	0.68	0.66	-2.49	0.52	0.58	12.50
MeanSWTL (TL)	2.23	2.18	-2.42	2.11	1.92	-8.92	2.23	2.21	-0.84	2.11	2.00	-4.94
MeanShortChn (Chain)	1.89	1.85	-1.95	1.82	1.70	-6.74	1.89	1.88	-0.29	1.82	1.76	-3.57
FracOmniv (Omn)	0.64	0.66	2.29	0.69	0.61	-11.96	0.64	0.63	-2.49	0.69	0.64	-6.62
FracCannibal (Can)	0.28	0.28	-0.12	0.22	0.22	1.56	0.28	0.29	3.40	0.22	0.19	-12.08
CharPathLen (Path)	1.78	1.73	-2.48	1.79	1.77	-0.83	1.78	1.77	-0.13	1.79	1.80	0.90
MeanClusterCoeff (Clust)	0.30	0.27	-12.94	0.24	0.28	15.33	0.30	0.30	-0.80	0.24	0.26	6.83
ResourceCount	32.76	19.12	-41.65	11.06	6.24	-43.62	32.76	31.00	-5.39	11.06	7.12	-35.64
ConsumerCount	32.00	19.24	-39.89	11.65	6.76	-41.92	32.00	30.12	-5.88	11.65	8.35	-28.28

4.4. Discussion

This work shows just how important it is to add a context to extinction exercises using food web networks. Firstly, it was showed that temperate and tropical webs have similar robustness to species loss based on thermal vulnerability. Secondly, it was showed that if only thermally vulnerable species are removed, then the high vulnerability of tropical food webs is revealed. It can then be concluded that tropical webs are not structurally vulnerable to warming *per se*, but they are more likely to have structural changes because they encompass a much higher number of vulnerable species.

Although, robustness was similar between temperate and tropical webs, the number of networks properties affected was higher in tropical webs, indicating that their structure was more deeply affected by species loss. In the heatwave exercise, there was no change in network properties in the temperate webs, when thermally vulnerable species were removed. The species removed were very few, so this was not surprising. In the tropical webs 10 network properties were altered, meaning that heatwaves can impact the organization of tropical intertidal rock pool webs. A decrease occurred in chain – the mean number of links in every possible food chain connecting top species to basal species – this implies that disturbance is more likely to rapidly travel throughout the web through predator−prey links (Williams et al. 2002), thus it an important indication that the web remaining after the heatwave is more fragile than before that disturbance.

This work gives an important contribute to the debate on whether the temperate or the tropical regions are more vulnerable to climate warming (Ghalambor et al., 2006; Tewksbury et al., 2008). Several authors pointed that although warming is predicted to be slower in the tropics, this region should, however, be more vulnerable than the temperate region because most of its species evolved in a thermally stable environment and thus have low acclimation capacity (Stillman, 2003, Hoffman & Todgham, 2010; Pörtner & Peck 2010; Somero, 2010, Vinagre et al. 2016, 2018). Moreover, tropical species have been shown to be living closer to their upper thermal limits and, thus, be living with lower thermal safety margins (Vinagre et al., 2019).

Vinagre et al. (2018) highlighted the particular vulnerability of tropical intertidal rock pools, that act as ecological traps due to the high temperatures they attain during heatwaves. Furthermore, many of the organisms who live in these pools are early-life stages seeking nursery areas, so the lifecycles of such species may be disturbed (Dias et al., 2016; Vinagre et al., 2018).

The present study shows the importance of taking the next step in the complexity scale, going from individual species vulnerability analyses to food web networks. However, it is important to acknowledge, that this first step in understanding structural changes in food webs due to warming produces conservative results and can be considered a best-case scenario (like all previous purely topological approaches). It underestimates the effects of each extinction, since it only allows bottom-up effects (secondary extinctions only occur when a predator loses all its prey species) (Curstdotter et al., 2011). Top-down extinction cascades are likely to also occur and have been widely observed in nature (e.g. Paine, 1966; Elmhagen & Rushton, 2007).

On the other hand, this approach can overestimate the number of secondary extinctions since it does not consider the ability of predators to alter their diet by switching prey (diet plasticity). Which is one of many possible types of compensatory dynamics in ecosystems (e.g. Brown et al., 2001). If a species easily changes its diet this will increase the robustness of the web while specialist species decrease the overall robustness.

According to Dunne et al. (2002b), it is necessary to remove about 40-50% of the species to collapse a food web network. On the realistic exercise conducted here, using present-day maximum habitat temperatures, on average 20% of species were removed in tropical webs. While this is probably too low to collapse the food web network, it is nonetheless a considerable amount. It is reasonable to assume that further warming, caused by global change, will eventually drive this value near the network collapse point. This will not mean the species inhabiting tropical pools will go extinct, since they also occur in colder subtidal waters, but intertidal rock pools could lose its ability to harbour complex food webs due to the harshness of their thermal conditions. They may also lose their refuge, feeding ground and nursery functions, which are very important for many small coastal organisms, especially transient fish early life-stages (Dias et al., 2016; Mendonça et al., 2019).

Our work shows for the first time that tropical food webs are more vulnerable to climate warming than temperate food webs. They are not more susceptible in structural terms, but they encompass more species likely to be lost due to warming in present conditions. Such a loss will likely result is food web network structure alterations. This way, the evidence supporting the relative higher vulnerability of tropical ecosystems mounts, highlighting the need to prioritize research and environmental management actions in tropical ecosystems.

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CHAPTER 5

Seasonal Variation in the Food Web Network Structure of Intertidal Rock Pools

Mendonça, V., Cereja, R., Dias, M., Flores, A.A:F., Vinagre, V. (submitted).

ABSTRACT

The seasonal variation of food web network structure of intertidal rock pools is unknown. In fact, no study ever tried to follow the seasonally changing food web network structure, in any ecosystem. Here, 32 intertidal rock pools were sampled in autumn, winter, spring and summer to assemble and compare their food web network structure. Marked seasonal changes in food web network structure were not detected. No seasonal variation was detected in the proportion of top, intermediate and basal species, as well as on the proportion of herbivores, omnivores and cannibal species, revealing a stable basic web topology throughout the year. The food web networks encompassed more taxa in summer and autumn than in spring and winter, mostly due to an increment in macroalgal species and transient marine fishes. Connectance was lower in summer and autumn which may mean lower network robustness. Mean shortest path length was higher in spring and summer, which may counterbalance the lower connectance in summer. Anemones and resident fish were important predators in the webs, but with little seasonality associated. It was detected that some species, albeit rare, when occurring, always occupy top trophic levels. Among them are highly valuable commercial species, such as the octopus that occurs mostly in autumn and winter and is a target of coastal fisheries in pools.

Keywords: seasonality, network structure, trophic interactions, rocky shore

5.1. Introduction

Food webs are descriptions of the trophic interactions between organisms in a biological community. These descriptions help the understanding of aspects of ecosystem structure, function and population dynamics (Pimm et al., 1991; Winemiller & Polis, 1996; de Ruiter et al., 2005; Winemiller & Layman, 2005). One of the main goals of food web studies has been to obtain empirical descriptions of different ecosystems in pursuit of general patterns or rules (Schoener, 1989; Cohen et al., 1993). Comparative studies showed ecosystems (i.e. estuarine, marine, stream, lake, terrestrial, tide pools) share similar general properties of complex food web network structure (Williams & Martinez, 2000; Camacho et al., 2002; Dunne et al., 2004; Bascompte J., 2009; Vinagre & Costa, 2014; Mendonça et al., 2018).

The great majority of food web studies group species and interactions recorded within a habitat over a relatively long period of time (Winemiller, 1990; Hall & Raffaelli, 1991; Martinez, 1991; Polis, 1991) or focus on a single point in time, neglecting seasonal dynamics, although it is broadly accepted that ecosystems are highly heterogeneous in space and in time (Kolasa & Rollo, 1991; Levin, 1992; Stewart et al., 2000; Vinagre et al., 2012; Vinagre & Costa, 2014). This lumping may obscure significant temporal variations in network structure, especially in highly variable habitats (Kitching, 1987; Closs & Lake, 1994, Vinagre et al., 2012).

Although the interest in studies and analysis of food web structure has increased, temporal dynamics remain a little studied feature in food web ecology. Some studies have reported that the structure of the food web may change due to intra-annual variability in freshwater communities (Closs & Lake, 1994; Tavares-Cromar & Williams, 1996; Thompson & Townsend, 2000; Peralta-Maraver, 2017) and tidal estuaries (Akin & Winemiller, 2006; Vinagre et al., 2012). To the best of our knowledge no study ever followed the food web network structure in the same biological system, throughout the year, to uncover its seasonality.

Studies in the rocky intertidal zone suggest that much of the structure of local communities depends on environmental conditions (Underwood et al., 1983; Brosnan, 1992; Menge & Sutherland, 1976, 1987). Some species of algae show marked seasonal changes in pattern of distribution, this variability varies from complete disappearance/appearance to changes in the upper limit of distribution (Underwood, 1981; Hartnoll &

Hawkins, 1985). Hot summers, cause macroalgae and invertebrates' deaths and mobile species retreat lower on the shore and take refuge in crevices (Lawson, 1966; John et al., 1992; Kaehler & Williams, 1996; Nagarkar & Williams, 1999; Williams et al., 2000; Menge & Lubchengo, 1981). In winter, macroalgae flourish and mobile invertebrates extend their vertical range and their foraging periods (Banaimoon, 1988; Murthy et al., 1989; Williams & Morritt, 1995; Williams et al., 2000; Menge & Lubchengo, 1981).

In temperate intertidal rock pool ecosystems, the composition and diversity of biota undergo seasonal changes (Metaxas & Scheibling, 1993). The amplitude of daily fluctuations of temperature, salinity and pH vary seasonally (Ganning, 1971; Metaxas & Scheibling, 1993). These fluctuations vary with the volume, surface area and depth of the pool, as well as its height on the shore, degree of shading, drainage pattern and exposure to waves and splash (Metaxas & Scheibling, 1993), which makes each rocky intertidal pool unique in its physical regime, since intertidal rock pools cannot be similar in all these characteristics.

Intertidal rock pools' community may exhibit seasonal variations in the abundance of microalgae, with a maximum in spring and minimum in summer (Aleem, 1950; Dethier, 1982). Macroalgae abundance also varies seasonally although this variation is species-specific (Underwood & Jernakoff, 1984), i.e. some species are present throughout the year, such as *Ulva lactuca* (Linnaeus, 1753) (Femino & Mathieson, 1980), and others species peak at different times of the year (Wolfe & Harlinm, 1988a; b). Fish that are either transient or resident species in intertidal rock pools show seasonal changes in abundance (Thompson & Lehner, 1976; Grossman, 1982, Yoshiyama et al., 1986; Moring, 1990). Some transient fish species are absent in winter and peak in numbers in spring or summer (Dias et al., 2014, 2016). These transient fish are mostly early life-stages seeking refuge and prey (Dias et al., 2014, 2016; Mendonça et al., 2019). Recent studies have shown that shrimp populations vary seasonally, peaking in spring and summer, although some species are present throughout most of the year, such as *Palaemon elegans* (Rathke, 1837) in the coast of Portugal (Vinagre et al., 2015).

The seasonal complex structure of food webs and their topological network properties has never been addressed for intertidal rock pools. This study aims to assemble highly resolved food web networks for an important number of intertidal rock pools and follow their seasonal variation in food web network properties, in a temperate region of the Northeast Atlantic, the west coast of Portugal. To the best of our knowledge this is the first time that any marine food webs have been followed throughout a whole year, with sampling performed in the exact same intertidal rock pools at each season for the assemblage of highly resolved food web networks. Members of each web were identified to species and lumping of species was avoided wherever possible. Food webs were built with an emphasis on standardisation of effort and rigorous attention to taxonomic detail to minimize the methodological problems observed in similar work.

5.2. Materials and methods

5.2.1. Study area

Two sites were chosen in the west coast of Portugal, distanced approximately 65 km, in these sites two beaches were targeted: Lourinhã (beach A - Paimogo - 39°17'11.4"N 9°20'23.7"W and beach B - Peralta 39°14'28.9"N 9°20'36.8"W) and Cascais (beach A – Cabo Raso - 38°42'38.2"N 9°29'09"W and beach B – Raio Verde - 39°17'11.4"N 9°20'23"W) (Fig. 1). At each beach, two replicate points were chosen, and four intertidal rock pools are sampled at each point (Fig 5.1). A total of 32 rock pools were sampled in each season (autumn 2015 - summer 2016). Sampling took place during low tides, always in same rock pools in each season. The study area is exposed to northwest oceanic swell and affected by strong hydrodynamics. The tidal regime is semidiurnal with tides that can range up to 4 m.

Figure 5.1. Location of the study area in west coast of Portugal. Indication of the beaches and the location of the replicate sampling points within each beach. Aerial photos adapted from Google Earth.

5.2.2. Sampling

All sampled intertidal rock pools were located at the lower intertidal, with a minimum depth of 0.19 m and a maximum depth of 0.41 m and surface area (estimated from scaled digital photographs using the software ImageJ) ranging from 0.16 m^2 to 14.69 m^2 (Table 5.1). The distance from the pool to the coastline and its elevation, i.e. the vertical distance from the mean sea level, was also registered (Table 5.1 and see Table SM5.1 in annex 4 for the main pool characteristics in each season). Water temperature (± 0.1°C) and salinity (± 1‰) was manually determined with a digital thermometer and a salinity meter (Fig 5.2).

Table 5.1. General description of the study beaches (averages obtained from the average pool characteristics in each season)

Beach	Site	Coordinates	Nº pools	Area (m^2)	Average depth (m)	Distance to coastline (m) (mean)	Elevation (m) (mean)
Cabo Raso	1	38°42'38.2"N 9°29'09.9"W	4	1.68	0.20	7.68	0.46
	2	38°42'36.0"N 9°29'09.9"W	4	4.63	0.27	13.58	0.49
Raio Verde	1	38°42'07.5"N 9°28'30.4"W	4	3.28	0.32	11.06	0.47
	2	38°42'06.7"N 9°28'28.1"W	4	1.72	0.27	19.24	0.32
Paimogo	1	39°17'11.4"N 9°20'23.7"W	4	4.84	0.29	17.28	0.48
	2	39°17'11.4"N 9°20'27.9"W	4	2.03	0.28	10.27	0.48
Peralta	1	39°14'28.9"N 9°20'36.8"W	4	6.88	0.41	10.23	0.29
	2	39°14'30.5"N 9°20'35.8"W	4	3.75	0.19	12.64	0.51

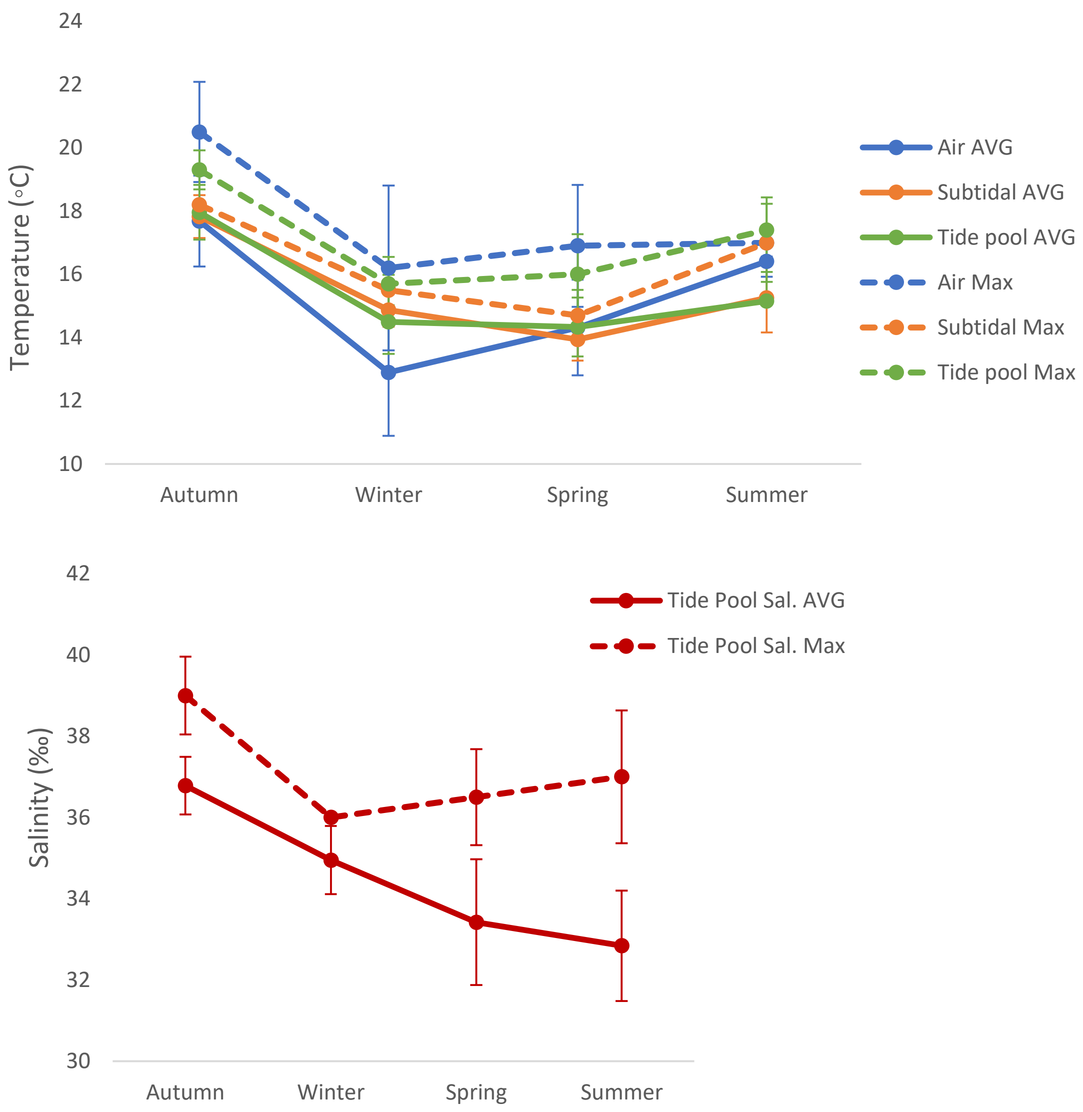

Figure 5.2. Average (AVG) and maximum (Max) temperatures measured in the subtidal, tide pools and outside air and average (AVG) and maximum (Max) salinities measured in the tide pools, during the seasons (2015/6).

At each intertidal rock pool, the substrate cover was registered, sediment samples were collected (50 ml) whenever the pool presented sediment at the bottom and three quadrats of 25 cm^2 of rock pool surface were randomly scrapped to bare rock with a flat chisel with 5 cm on its scrapping edge. Sediment and scraping samples were collected into a plastic bag very carefully to minimize losses, preserved in alcohol 70% with Bengal rose and transported to the laboratory, where all organisms were identified with a stereomicroscope, measured (Lt), precision of 0.01 mm and weighed (Wt), precision of 0.0001 g. All macro-organismos present in the pools were identified *in situ*, measured (total length with a precision of 1 mm) and weighed (wet weight with a precision of 0.01 g). Fish, shrimp and crabs were collected with hand nets (1 mm mesh) and macroalgae, sponges, cnidarians, polychaetes, molluscs and echinoderms were collected by hand. Whenever there were doubts in the identification taxonomical, the organisms were taken to the laboratory and identified using identification keys and contacting taxonomy experts.

Microorganisms were included in food webs as a group, divided into zooplankton and phytoplankton due to low definition of predator's diet. Detritus was considered a food web node in all pools.

The lists of species compiled for each pool were used to build highly resolved predator–prey matrices, based on a literature search on their diets (see Mendonça et al. (2018) for diet references).

5.2.3. Network structure of food webs

The matrices describing predator-prey relations were used as input on the software Network3D (Williams, 2010) to assemble highly defined food webs. The networks analysed were based on trophic species. Trophic species are taxa that share a similar set of prey and predators (Briand & Cohen, 1984). The use of trophic species is a convention in structural network studies of food webs, they reduce methodological biases of uneven resolution among food webs (Williams & Martinez, 2000).

Using the software Network3D (Yoon et al., 2004; Williams, 2010) eighteen network properties were calculated for each food web (Table 5.2): number of trophic species (S), links per species (L/S), connectance (C, where $C = L/S^2$), T (percentage of top predators), I (percentage of intermediate species), B (percentage of basal species), Can (percentage of cannibals), Omn (percentage of omnivores), H (percentage of herbivores and detritivores), Resource count (number of resources in the food web), Consumer count (number of consumers in the food web), TL (mean short-weighted trophic level), Chain (mean number of links in every possible food chain), Path (characteristic path length), GenSD (standard deviation of mean generality), VulSD (standard deviation of vulnerability), LinkSD (normalized standard deviation of links) and Clust (clustering coefficient).

Table 5.2. Definition of the food web properties calculated.

Food-web properties		Description
Number of trophic species	S	Number of species in the food web after being converted into a trophic web
Links per species	L/S	Number of pred/prey links per species
Connectance	C	Proportion of actual trophic links to all possible links (L/S^2)
Top species	T	Species with prey and not predators or parasites
Intermediate species	I	Species with both predators and prey
Basal species	B	Species with predators and no prey
Herbivores plus detritivores	H	Species who prey on primary producers
Cannibals	Can	Species which prey on their own species
Omnivores	Omn	Species with food chains of different lengths, where a food chain is a linked path from a nonbasal to a basal species
Resource count		Count of all species that serve as resources in the food web
Consumer count		Count of all species that serve as consumers in the food web
Trophic level	TL	Trophic level averaged across taxa
Mean food chain length	Chain	Mean number of links in every possible food chain or sequence of links connecting top species to basal species
Mean shortest path lenght	Path	The mean shortest set of links between species pairs
Generality standard deviation	GenSD	Resources per taxon, how many prey items a species has
Vulnerability standard deviation	VulSD	Consumers per taxon, how many predators a species has
Normalized standard deviation of links	LinkSD	Links per taxon
Clustering coefficient	Clust	The mean shortest set of links between species pairs

5.2.4. The niche model

The predictive success of the niche model in terms of food web properties was estimated for each intertidal rock pool food web (Williams & Martinez, 2000). This model has two input parameters: the number of trophic species (S) and connectance (C). It gives each species (*i*) a randomly drawn 'niche value' (n_i) from the interval (1,0), and each species is then constrained to feed on all prey species within a range of values (r_i) of which randomly chosen centre (c_i) is less than the consumer's niche value. In this model allows up to half a consumer's range to include species with higher niche values than the consumer, thus allowing looping and cannibalism. The consumer is forced to feed on all species within its feeding range (r_i).

Monte Carlo simulations were used, for each food web, to generate 1000 niche model webs with same S and C as the original web, allowing the estimation of model mean and standard deviation for each of the 18 network properties. Whenever the normalized error (raw error divided by model SD) between the empirical property and the mean model value for that property was within ±1 model SD, the model was deemed to be a good fit to the empirical data (Dunne et al., 2013; Mendonça & Vinagre, 2018; Mendonça et al., 2018; Vinagre et al., 2019). The previous calculations were performed in the software Network3D. The percentage of niche model errors was estimated for each pool and the mean percentage was estimated for each season. A mean percentage of niche model errors < 30% is considered a good fit (Dunne et al., 2004).

5.2.5. Statistical analysis

The food web network properties analysed were compared between seasons, significant differences among the network properties and the percentage of niche model errors, for the seasons were investigated with a one-way ANOVA (factorial analyses of variance), followed by Tukey post-hoc tests. Prior to these tests, normality and homoscedasticity were confirmed. Each property was tested separately and a significance level of 0.05 was considered in all test procedures. All statistical analyses were carried out using the Statistica software (version 12.0, StatSoft Inc., USA).

5.3. Results

Higher temperatures occurred in autumn, not summer, for air, pool and subtidal waters (Fig. 5.2). Maximum air, pool and subtidal water temperatures followed a similar pattern (Fig. 5.2). The maximum temperature recorded in pool waters was 19.3ºC in autumn, while the minimum was 15.5◦C in winter.

The data assembled for the intertidal rock pool food webs of the study sites resulted in lists ranging from 34 to 71 taxa in the autumn, 29 to 65 taxa in the winter, 27 to 59 taxa in spring, and 40 to 67 taxa in the summer. These taxa corresponded to 18 to 50 trophic species in the autumn, 13 to 42 trophic species in the winter, 15 to 36 trophic species in the spring, and 20 to 45 trophic species in the summer (Fig. 5.3). The Network3D analyses shows a visual representation of the complex food webs assembled for each season including all species (see Table SM5.2 in annex 4 for list of all taxa identified).

Some significant differences among seasons were detected for some food web network properties. Number of taxa (S) was higher in the autumn and summer than in the winter and spring (Fig. 5.4, Table SM5.3 in annex 4). This pattern was similar for GenSD, Resource count, and Consumer count (Fig. 5.4, Table SM5.3 in annex 4). Autumn presented higher links per species (L/S) than the other seasons (Fig. 5.4, Table SM5.3 in annex 4). Connectance (C) was higher in the winter and spring than in the autumn and summer (Fig. 5.4, Table SM5.3 in annex 4). The consumers per taxa (VulSD) was higher in the summer than the other seasons and similar pattern was observed in the links per taxon (LinkSD) and the mean shortest path length between species pairs (Path) (Fig. 5.4, Table SM5.3 in annex 4). No differences between seasons were observed in the species types present in the food webs, namely in the percentage of top (T), intermediate (I), basal (B), herbivores (H), omnivores (Omn) and cannibals (Can) species (Fig 5.4, Table SM5.3 in annex 4). Also no differences were found in trophic level (TL), sequence of links connecting top species to basal species (Chain) and clustering coefficient (Clust) (Fig. 5.4, Table SM5.3 in annex 4).

The taxa most frequently in the top 3 highest trophic level (TL) were the same in all seasons, a cnidarian species *Anemonia sulcata* (Pennant, 1777), Nematoda and a marine fish species *Lipophrys pholis* (Linnaeus, 1758) (Table 5.3). The highest trophic level varied between 2.4 and 3.0 in autumn, between 2.5 and 2.9 in the winter, between 2.6 and 2.9 in spring and between 2.6 and 2.9 in summer.

The taxa most frequently found in the top 3 in terms of generality varied among the seasons, with the fish *Lipophrys pholis* and the cnidaria *Anemonia sulcata* present in all seasons, and the fish *Coryphoblennius galerita* (Linnaeus, 1758) only present in autumn and spring while in winter and summer the third species in the top 3 is the anemone *Actinia equina* (Linnaeus, 1758) (Table 5.3). The highest values of generality varied between 1.7 and 8.0 in autumn, between 1.5 and 4.6 in the winter, between 1.4 and 4.7 in spring and between 1.5 and 4.7 in summer.

The taxa most frequently in the top 3 of highest vulnerability were "detritus", although this is not a taxon, this node was always among with highest connectivity on all seasons (Table 5.3). Zooplankton and Macroalgae was also present in all season (Table 5.3). The highest values of vulnerability varied between 2.0 and 6.0 in autumn, between 1.9 and 6.0 in the winter, between 1.8 and 5.9 in spring and between 2.2 and 6.7 in summer.

The taxa most frequently found in the top 3 in terms of connectivity were the same in all seasons analysed, with detritus, the fish *Lipophrys pholis* and the zooplankton, except in the winter where fish *Coryphoblennius galerita* was more important than zooplankton (Table 5.3). The highest values of connectivity varied between 1.5 and 4.5 in autumn, between 1.4 and 3.0 in the winter, between 1.5 and 2.9 in spring and between 1.8 and 3.4 in summer.

Unusual taxa that, when present in intertidal rock pools, occupy most frequently the top 3 highest trophic level in all seasons were the *Octopus vulgaris* (Cuvier, 1797), the crab *Necora puber* (Linnaeus, 1767) and the fish *Lepadogaster lepadogaster* (Bonnaterre, 1788), except in summer where the crab *Carcinus maenas* (Linnaeus, 1758) was present instead of *N. puber* (Table 5.4).

Mean niche model percentage of errors varied between 16% (spring) and 23% (autumn) (Fig. 5.5). The value was significantly higher for the intertidal rock pools surveyed in autumn, in comparison to all other seasons, with the exception of summer ($p < 0.002$; Fig. 5.5).

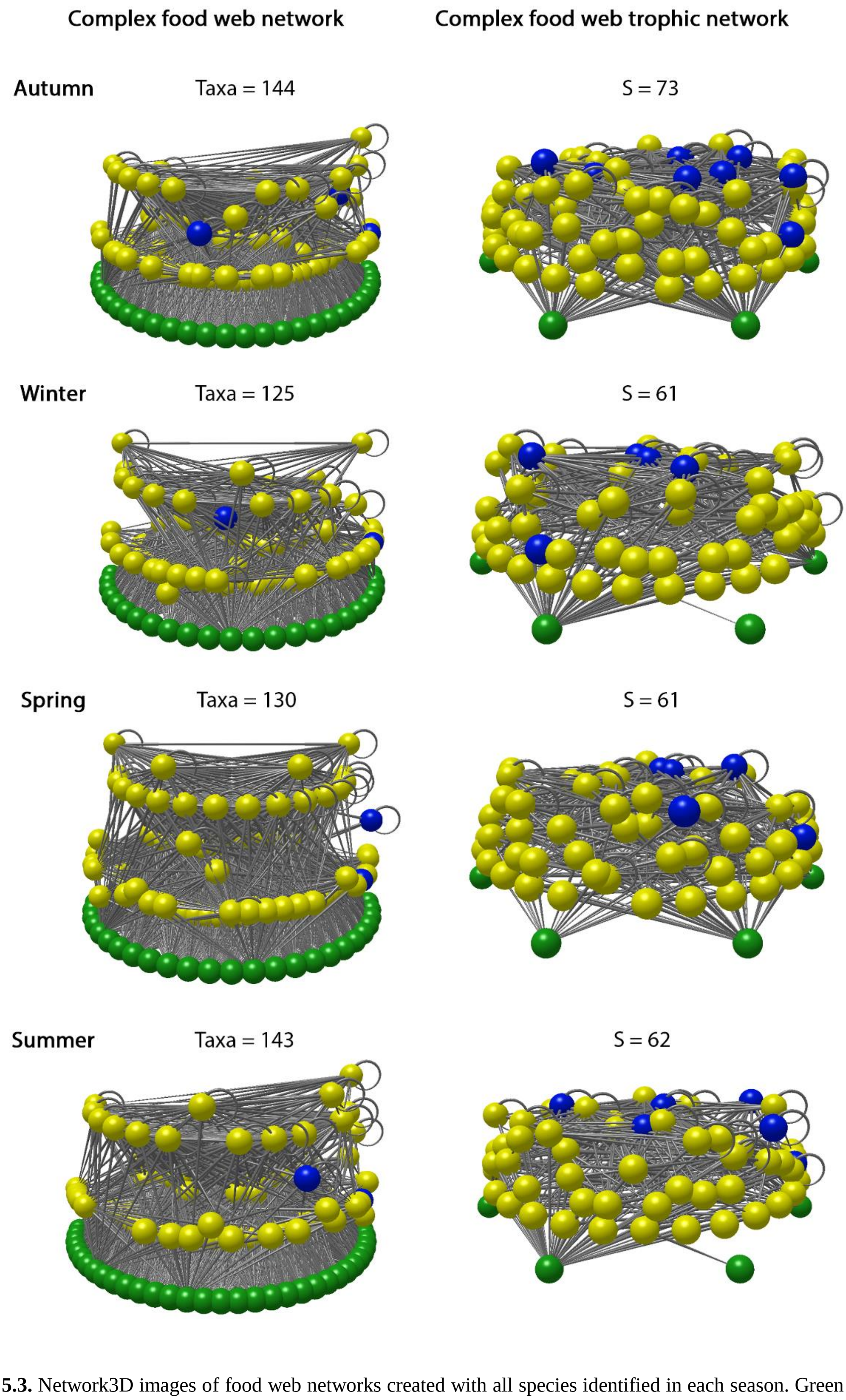

Figure 5.3. Network3D images of food web networks created with all species identified in each season. Green nodes = basal taxa; yellow nodes = invertebrates; blue nodes = vertebrates. On the left complex food web networks are depicted, on the right are the trophic species versions of the same food webs. Trophic species are groups of taxa whose members share the same set of predators and prey and are thus aggregated in single nodes.

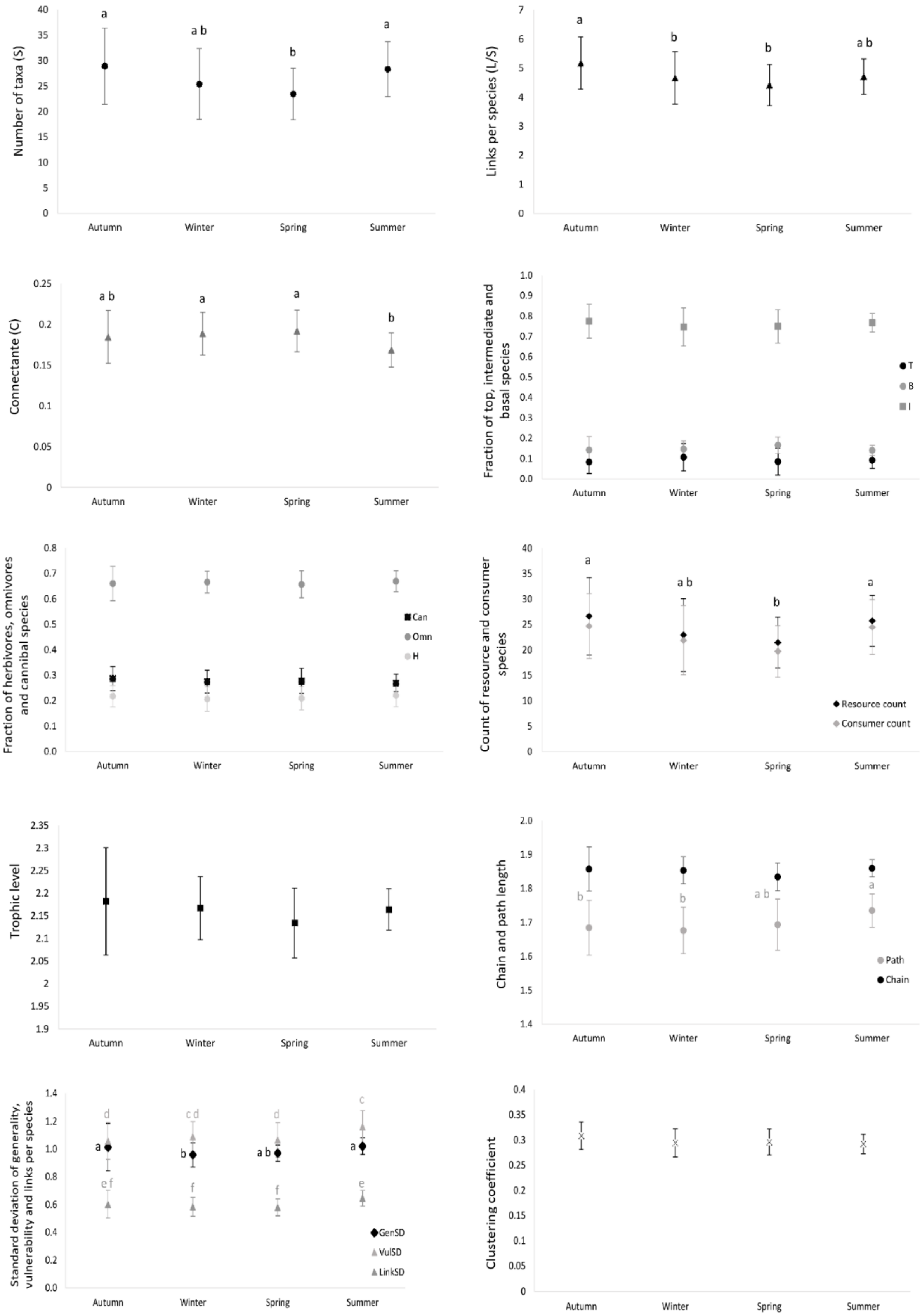

Figure 5.4. Variation of the main food web properties across the different seasons (dots indicate mean values, bars indicate standard deviation, letters indicate significant differences)

Table 5.3. Taxa most frequently in the top 3 of highest trophic level (short-weighted), generality, vulnerability and connectivity in the food webs analysed.

	Taxa most frequently in the top 3 highest **trophic level**	Number of webs where the taxa were in the top 3 highest **trophic level**	Taxa most frequently in the top 3 highest **generality**	Number of webs where the taxa were in the top 3 highest **generality**	Taxa most frequently in the top 3 highest **vulnerability**	Number of webs where the taxa were in the top 3 highest **vulnerability**	Taxa most frequently in the top 3 highest **connectivity**	Number of webs where the taxa were in the top 3 highest **connectivity**	Total webs analysed
Autumn									32
	Anemonia sulcata (cnidaria)	23	*Lipophrys pholis* (fish)	24	Detritus	32	Detritus	31	
	Nematoda	14	*Anemonia sulcata* (cnidaria)	23	Zooplankton	32	*Lipophrys pholis* (fish)	23	
	Lipophrys pholis (fish)	11	*Coryphoblennius galerita* (fish)	18	Macroalgae	31	*Coryphoblennius galerita* (fish)	17	
Winter	*Anemonia sulcata* (cnidaria)	21	*Anemonia sulcata* (cnidaria)	21	Detritus	32	Detritus	32	32
	Nematoda	14	*Lipophrys pholis* (fish)	20	Zooplankton	31	*Lipophrys pholis* (fish)	20	
	Lipophrys pholis (fish)	10	*Actinia equina* (cnidaria)	11	Macroalgae	26	Zooplankton	19	
Spring	Nematoda	22	*Anemonia sulcata* (cnidaria)	22	Zooplankton	32	Detritus	32	32
	Anemonia sulcata (cnidaria)	20	*Lipophrys pholis* (fish)	22	Detritus	31	*Lipophrys pholis* (fish)	22	
	Lipophrys pholis (fish)	15	*Coryphoblennius galerita* (fish)	14	Macroalgae	29	Zooplankton	18	
Summer	Nematoda	24	*Lipophrys pholis* (fish)	29	Detritus	32	Detritus	32	32
	Anemonia sulcata (cnidaria)	21	*Anemonia sulcata* (cnidaria)	21	Zooplankton	32	*Lipophrys pholis* (fish)	29	
	Lipophrys pholis (fish)	17	*Actinia equina* (cnidaria)	9	Macroalgae	30	Zooplankton	17	

Table 5.4. Unusual species in food networks but when present they are in the top 3 highest trophic level (short-weighted) and number of webs where they occur.

Unusual taxa most frequently in the top 3 highest trophic level							
Autumn		***Winter***		***Spring***		***Summer***	
Necora puber (crab)	8	*Octopus vulgaris* (octopus)	7	*Aeolidia papillosa* (nudibranchia)	1	*Carcinus maenas* (crab)	4
Octopus vulgaris (octopus)	7	*Necora puber* (crab)	6	*Lepadogaster lepadogaster* (fish)	3	Nemertea	4
Syngnathus acus (fish)	4	*Lepadogaster lepadogaster* (fish)	3	*Necora puber* (crab)	2	*Lepadogaster lepadogaster* (fish)	2
Aeolidia papillosa (nudibranchia)	3	*Syngnathus acus* (fish)	2	Nemertea	3	*Octopus vulgaris* (octopus)	2
Lepadogaster lepadogaster (fish)	3	*Felimida purpurea* (nudibranchia)	1	*Octopus vulgaris* (octopus)	5	*Diaphorodoris papillata* (nudibranchia)	1
Hypselodoris sp. (nudibranchia)	1	Turbellaria	1	Turbellaria	1	Turbellaria	1
Maja squinado (crab)	1						
Symphodus sp. (fish)	1						

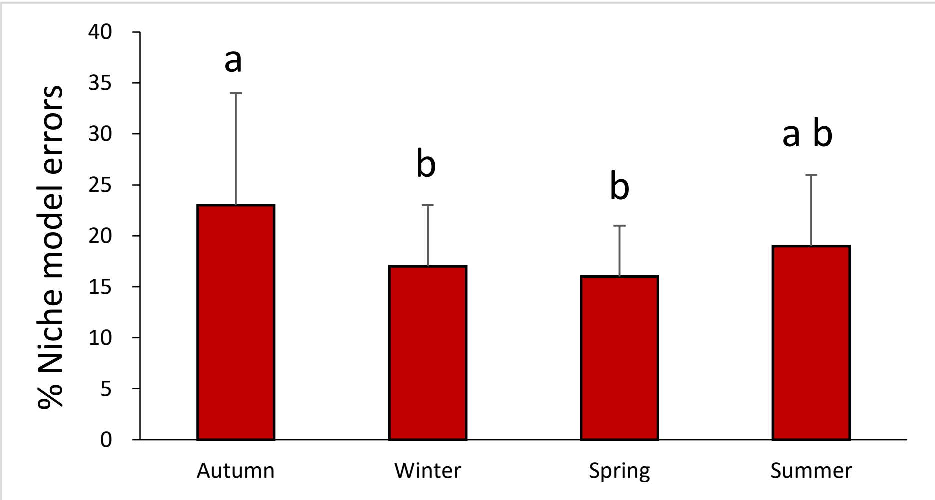

Figure 5.5. Percentage of niche model errors for 18 network structure properties that are greater than │1│. Error bars indicate standard deviation and the letters indicates a significant differences.

5.4. Discussion

Seasonality in thermal conditions was not very marked in these intertidal rock pools, with temperatures rarely exceeding 20ºC and rarely reaching values lower than 14ºC. Higher temperatures in air, pools and subtidal waters, occurred in autumn, not summer. This happens due to the particular hydrographic characteristics of the Portuguese coast, which encompass summer upwelling of cold waters due to persistent northerly winds, which typically relax in late summer and are absent in Autumn (Fiúza, 1983; Haynes et al., 1993; Smyth et al., 2001) (Fig. 5.2).

Seasonality in the total number of species is not high, with a peak in Autumn and Summer and almost no difference in numbers between Autumn (n = 141) and Summer (n = 140). Numbers were lowest in winter (n = 122) and increased in spring (n = 127), as expected, associated to the increasing photoperiod from winter to spring that allows an increase in primary production (Michaletz et al., 2014). The additional species, that were not present in winter, but increased in spring until reaching the summer/autumn peak were mostly macroalgae species and transient fishes, like *Diplodus sargus* (Linnaeus, 1758), *Diplodus cervinus* (Lowe, 1838), *Conger conger* (Linnaeus, 1758), *Liza ramada* (Risso, 1827) and *Symphodus melops* (Linnaeus, 1758). Early life-stages of transient fish occurring, sometimes in high densities, in intertidal rock pools have been reported by Dias et al. (2014, 2016), in the same area. Several authors have hypothesized that the use of intertidal pools during early ontogeny may be favourable for growth, fitness and survival (Thompson & Lehner, 1976; Moring, 1986, 1990; Mahon & Mahon, 1994; Macpherson, 1998). The three-dimensional complexity of pools also offers refuge from larger predators, especially during ebb tide, since large predators remain in subtidal waters (Metaxas & Scheibling, 1993; Gibson, 1994; Mahon & Mahon, 1994). It is also believed that pool waters' higher temperatures (than subtidal waters) in spring and summer are beneficial for growth (Prochazka, 1996), however, in the present study such a temperature difference was not found between subtidal and pools waters. Recently, Mendonça et al. (2018) showed that transient juvenile fish use intertidal rock pools as preferential feeding grounds. These highly structured environments harbour abundant macroalgae and small invertebrates which are important food items for larval and juvenile fish (Beckley, 1985a; Moring, 1986; Amara & Paul, 2003; Cunha et al., 2007).

No seasonal variation was detected in the proportion of top, intermediate and basal species, as well as on the proportion of herbivores, omnivores and cannibal species. This means that overall the constitution of these food web networks is quite stable in its basic topology throughout the year.

Autumn and Summer present, not only higher number of taxa, but also higher links per species and lower connectance. Since connectance have been showed to be correlated with higher network robustness to species loss (Dunne et al., 2002, 2004), autumn and summer food web networks are likely to be less robust than the food web networks established in pools during winter and spring, despite having more species. Nevertheless, no seasonal differences were detected in chain length, so disturbance is not likely to affect many food web components through predator–prey links in a different way throughout the year (Williams et al., 2002).

The mean shortest path length (Path) within pairs of taxa (1.7) is slightly higher than previously determined for larger open marine systems (1.6) (Dunne et al., 2004), but lower than determined for other ecosystems (1.3-3.7) (Dunne et al., 2002b). This means that disturbance is likely to spread rapidly and widely throughout the food web network (Williams et al., 2002). Seasonality was detected for this food web network property, with Path being higher in Spring-Summer than in Autumn-Winter, possibly counterbalancing the lower connectance that occurs in summer.

The intertidal rock pool food web networks analyzed here fit the niche model well (mean error < 25% for all seasons), however the autumn food web displayed significantly lower fit to the niche model than the other seasons. The niche model predictive success was remarkably high (77-84%) and similar to previously found for intertidal rock pools (Mendonça et al., 2018). This predictive success rate is also similar to the 79% found for 7 non-marine food webs (Williams & Martinez, 2000) and the average 87% found for 3 marine food webs: the Benguela ecosystem off the coast of South Africa, a Caribbean coral reef ecosystem from the Puerto Rico - Virgin Islands shelf complex and a shelf ecosystem off the Northeast US (Dunne et al., 2004).

No seasonality was detected in the mean trophic level. Taxa most frequently in the top 3 highest trophic level were the same triad of species throughout the year, the anemone *A. sulcata,* Nematoda and the resident fish *L. pholis*. Although the prey of the Nematoda are just detritus and zooplankton in networks where Nematoda occurs they have no predators. The food chain established is Netamoda eating detritus and zooplankton, the later eating phytoplankton, establishing Nematoda as both a primary and a secondary consumer.

A. sulcata and *L. pholis* are also among the taxa most frequently in the top 3 of highest generality, throughout the year, meaning that they have the highest number of prey of all species. The fish *C. galerita* complete the top 3 of generality in autumn and spring, while in winter and summer another anemone completes this top 3, *A. equina*. This reveals the high importance of anemones as predators in these food webs, as well as that of resident fish.

Taxa most frequently in the top 3 of highest vulnerability, that is the taxa with more predators, were the same throughout the year, detritus, zooplankton and macroalgae, not surprisingly since this is where the webs are more agglomerated due to the general lack of detailed diet studies for their predators. They serve as food for most of the primary consumers. Higher taxa resolution at this level would probably reveal highly vulnerable and not so vulnerable species, particularly for macroalgae. Some macroalgae are likely to have a chemical composition that serve as defense against predation, like previously observed (Van Alstyne, 1988; Paul & Van Alstyne, 1992).

Taxa most frequently in the top 3 of connectivity, also did not change throughout the year. They were detritus, *L. pholis* and zooplankton, except for autumn when zooplankton was substituted by the resident fish *C. galerita*.

Some species are unusual, occurring in ≤ 8 webs in a total of 32, but when they occur, they occupy the top 3 highest TL. Autumn displays more such occurrences (8 species versus 6 in the other seasons). One important species among such occurrences is *Octopus vulgaris,* more frequent in autumn and winter and an important commercial species in the west coast of Iberia. Octopuses are fished in intertidal rock pools, being this the most valuable economic resource collected in these pools (Silva, 2009; Jereb et al., 2014).

N. puber, a commercially important crab in Europe, was also present in pools as a top-level predator, in all seasons except for summer. *Necora puber* has been described as one of the dominant epibenthic predators regulating the abundance and distribution of prey populations in rocky shore environments. It is known to consume limpets, chitons, mussels and barnacles (Kitching et al. 1959, Muntz et al. 1965, Aronson 1989, 1992). Beja (1995) showed that *N. puber* occurs all year round in rocky intertidal and subtidal habitats of the southwestern Portuguese coast and has minimal abundance in July and August. Also, Flores & Paula (2001) recorded the presence of *N. puber* in intertidal rock pools in the same area of this work.

Another highly valuable crab, the spider crab *Maja squinado* (Herbst, 1788), was also a top predator in pools, but it occurred only in summer. *M. squinado,* is a species of great commercial interest, occuring from the subtidal zone down to depths of 90 m (Kergariou, 1984; Le Foll, 1993). This species presents larger scale movements and seasonal migration along the coast occupying primarily deeper water in winter and shallower water in summer, this behavior is due to seasonal cycles in temperature and food availability (Hines et al. 1995). The presence of these species in tide pools confirms the seasonal migration pattern reported by Hines et al. (1995).

Various species of nudibranchs occupy the top predator's level when they occur in pools, but their occurrence is quite rare, typically occurring in only one pool in 32 in all seasons, except for autumn when they occurred in 3 pools out of 32. The fundamental ecological factor recognized for habitat selection by heterobranchs is the food supply (Clark, 1975; Todd, 1981; Sanvicente-Añorve et al., 2012). The species *Aeolidia papillosa* (Linnaeus, 1761) is cnidarian-eater (Hall & Todd, 1986; Betti et al., 2017) and was the most common species of nudibranchs in the present study, occurring in autumn and spring. The anemones *A. equina* and *A. sulcata* were present in all intertidal rock pools where the species *A. papillosa* occurred. Edmunds et al. (1974) reported that *A. papillosa* has a clear feeding preference for these actinian anemones. The species *Diaphorodoris papillata* (Portmann & Sandmeier, 1960) is a bryozoan-eater, while *Hypselodoris sp.* (Stimpson, 1855) and *Felimida purpurea* (Risso, 1831) are sponge-eaters (Coelho & Calado, 2010; Betti et al., 2017). The slow-growing massive sponges and fast-growing groups (hydroids, bryozoans, ascidians) are more abundant in the subtidal zone than in tide pools (Clark, 1975; Denny & Gaines, 2007). So, nudibranchs that feed on sponge and bryozoan possibly are absent or very rare in intertidal rock pools probably because of the lack of their prey.

The fact that marked seasonality in the food web topology was not detected, does not mean that these food webs do not suffer important seasonal changes in terms of species biomass and energy transfer. Future studies using allometric food web models taking biomass and species interaction strength into account should bring new insights into the seasonality of intertidal rock pool food webs. The present work exposes the basic topology of these webs and is, thus, a first step in this investigation.

5.5. Conclusions

This work showed for the first time that the food web network's topology of intertidal rock pools is not affected by marked seasonality. The proportion of top, intermediate and basal species, as well as the proportion of herbivores, omnivores and cannibal species was not affected by seasonality. Summer-Autumn webs have more species than winter-spring webs, mainly due to additional macroalgae and transient fish species. Anemones and resident fish were important predators in the webs, but with little variation in species composition throughout the year. Some species are relatively rare, but when they occur, they assume top trophic levels. Among them are highly valuable commercial species, such as the octopus that occurs mostly in autumn and winter and is a target of coastal fisheries in pools.

5.6. References

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CHAPTER 6

Do marine fish juveniles use intertidal rock pools as feeding grounds?

Mendonça, V., Flores, A. A., Silva, A. C., & Vinagre, C. (2019). Estuarine, Coastal and Shelf Science, 106255. DOI: 10.1016/j.ecss.2019.106255.

ABSTRACT

Several authors have put forward the hypothesis that juveniles of transient marine fish concentrate in intertidal rock pools, not only to benefit from refuge and higher temperatures, but also to use them as feeding grounds. However, there have been no attempts to test this. The feeding ecology of fish was studied in intertidal rock pools to evaluate the importance of these habitats as feeding grounds for non-resident juvenile fish. Fish were collected in 5 beaches in Southeastern Brazil. Juveniles of four species of non-resident fish were identified in the pools: *Abudefduf saxatilis* (Linnaeus, 1758), *Diplodus argenteus* (Valenciennes, 1830), *Eucinostomus melanopterus* (Bleeker, 1863) and *Odontesthes argentinensis* (Valenciennes, 1835). The most abundant species was *A. saxatilis*, followed by *E. melanopterus* and *D. argenteus*. The diet of those three species was characterized and correlated to the supply of potential food items in the pool where they were collected, in the surrounding intertidal and the nearest subtidal habitat. There was extensive diet overlap among species, largely explained by a generalized predation on copepods. The diet of all species was best correlated to the frequency of resources in tide pools, except for the diet of *E. melanopterus* which was equally correlated to the intertidal nearby habitat. The consistent similarity between stomach contents and prey availability inside the pool, observed for all species, indicates that intertidal tide pools are likely used as feeding grounds by juvenile stages of these transient fish.

Keywords: tide pools, juvenile fish, diet, stomach content, feeding habitats

6.1. Introduction

Some marine fishes are known to change habitat during their ontogeny, occurring in shallow waters during early life-stages and moving to greater depths as they grow (e.g. Beck et al., 2001; Gillanders, 2005). This is considered an adaptive response to avoid predation, reduce competition and enhance feeding opportunities (e.g. Lenanton, 1982; Boesch & Turner, 1984; Macpherson, 1998).

Nursery habitats have initially been described as areas where growth and survival of juveniles are enhanced (Gibson, 1994), or areas where juvenile fish concentrate and grow prior to first spawning (Steves et al., 1999). Later, Beck et al. (2001) defined nursery habitats as areas that provide higher survival, faster growth, higher densities and higher contribution of individuals to the adult habitats.

Several marine ecosystems have been classified as nursery areas for juvenile fish: estuaries (Wallace & Van Der Elst, 1975; Wallace et al., 1984, Costa & Bruxelas, 1989; Vinagre et al., 2008, 2010, 2012), coastal lagoons (Franco et al., 2006), salt marshes (Veiga et al., 2006), coral reefs (Nagelkerken et al., 2000), surf zone reefs (Berry et al., 1982), mangroves (Nagelkerken et al., 2000; Verweij et al., 2008) sandy beach surf zones (Lasiak, 1981, 1983) and intertidal rock pools (Dias et al., 2016).

Intertidal rock pools are the less studied potential nursery areas for fish. Several studies have been carried out on larvae and juveniles of some marine transient fish species (Gibson, 1982; Beckley, 1985a; Almada & Faria, 2004; Barreiros et al., 2004) and small resident fish (Almada et al., 1983, 1994; Almada & Santos, 1995; Faria & Almada, 2001) occurring in intertidal rock pools, but few studies investigated the nursery function of these habitats (Beckley, 1985b; Bennett, 1987; Gibson & Yoshiyama, 1999; Dias et al., 2014, 2016).

Resident tide pool fishes are mostly small-sized species of the families Gobiidae and Blenniidae (Horn et al., 1999), which exhibit specific morphological, physiological and behavioural adaptive traits that have been selected through evolution in this habitat (Arruda, 1990; Santos et al., 1994). However, intertidal rock pools are also important nursery areas for many different transient fish species, including scarids, sparids, pomacentrids, gerreids, atherinids, acanthurids, pomacanthids, chaetodontids, lutjanids, serranids and haemulids (Gibson, 1982; Mahon & Mahon, 1994).

Intertidal rock pools are often covered by dense vegetation canopy, offering great structural complexity and thus shelter against larger predators (Bennett & Griffiths, 1984; Gibson & Yoshiyama, 1999; Davis, 2000) and suitable food supply through the provision of vital invertebrates which are commonly preyed by juvenile fish (Beckley, 1985b; Moring, 1986; Amara & Paul, 2003; Cunha et al., 2007; Grossman, 1982). Also, the temperature within tide pools becomes higher than the nearby shallow-water habitat over low-tide periods, which may benefit metabolism and increase growth rates of early fish recruits during spring and summer (Haedrich, 1983; Gibson, 1994).

Intertidal rock pools are used as nursery areas for juvenile coastal fish species of high economic importance, such as *Diplodus sargus* (Linnaeus, 1758) (Garcia-Rubies, 1997; Dias et al., 2014, 2016). Because changes in the quality of this habitat will probably incur in poor recruitment and consequently a decrease of adult stocks (Riley et al., 1981; Haedrich, 1983; Miller et al., 1985), a better understanding of the relevance of this ecosystem to transient juvenile fish would therefore assist any environmental policies towards the conservation and protection of intertidal tide pool habitats (Gillanders & Kingsford, 1996; Yamashita et al., 2000; Beck et al., 2001; Gillanders, 2005; Dahlgren et al., 2006).

The objectives of this work are to characterize the diet of the most common transient fish species that occur in intertidal rock pools, and compare the food items that occur in fish stomachs with the prey available inside the pool, outside the pool and in the adjacent subtidal area, in order to evaluate the relative importance of intertidal rock pools in the feeding ecology of juvenile transient fish.

6.2. Material and methods

6.2.1. Study area

This study was conducted at five sites along a mostly rocky coastline in Southeastern Brazil (São Sebastião, São Paulo State, Fig. 6.1). In these sites the criteria followed for the selection of intertidal tide pools was that they must contain transient fish and have a size that allows the capture of all individuals, thus eight intertidal tide pools were selected. The tides in this area are semidiurnal with maximal variation of 1.3 m and the climate is wet tropical (Silva et al., 2005; Flores et al., 2015). The coastal landscape is composed of intertidal rocky platforms of basalt, and basal boulders, that punctually contain tide pools, mostly located in the lower intertidal at a height < 1 m. Subtidal shallow waters have the same rocky substrate and often also large patches of fine sand colonized by macrophytes. Intertidal platforms are fringed by dense Atlantic rainforest.

This study was conducted from January to February 2016, during periods of low tide. Each pool was visited 2 times, in consecutive spring tides that is, on consecutive days, in order to obtain a minimum number of fish for statistically research.

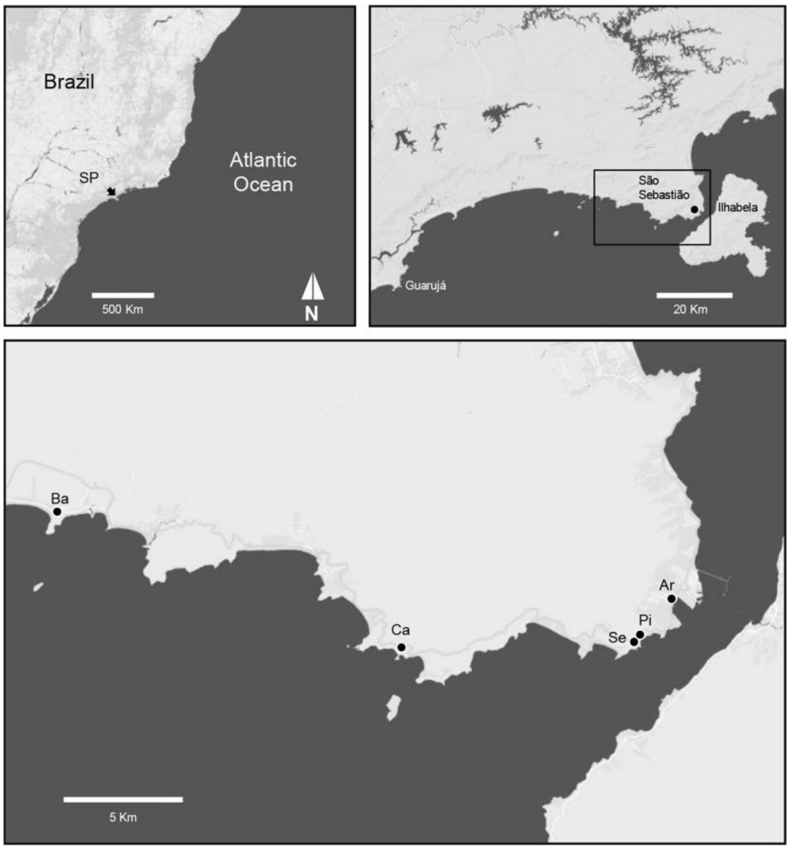

Figure 6.1. Location of the study sites along the coast of São Sebastião, SP (Southeastern Brazil). Ba: Baleia, Ca: Calhetas, Se: Segredo, Pi: Pitangueiras, Ar: Araçá.

6.2.2. Sampling

All sampled intertidal rock pools were located in the lower intertidal, the depth varied between 19 and 48 cm and their area, as estimated from scaled digital photographs using the software ImageJ, ranged between 28.2 and 25 m^2 (Table 6.1). Water temperature (± 0.1 °C) and salinity (± 1‰) was manually determined with a digital thermometer and a salinity meter (water: 28.2–33.2 °C, salinity: 34.5–37.2 ‰) (Table 6.1).

Table 6.1. Depth, approximate area, temperature and salinity of each studied tidal pool and number of transitional fish species present.

Tide pool	Locations	Depth (cm)	Area (m^2)	T (°c)	Salinity (‰)	*Abudefduf saxatilis*	*Diplodus argenteus*	*Eucinostomus melanopterus*	*Eucinostomus melanopterus* post-larvae	*Odontesthes argentinensis*
P1	Araçá	25	15	29.5	36			4	38	5
P2	Baleia	30	8	30.8	35.5	10				
P3	Baleia	30	18	29.3	34.5	10	28			
P4	Pitangueiras	22	25	29.1	37.2	9	9	58	141	
P5	Calhetas	19	8	30	36.5	5				
P6	Calhetas	48	10	29.9	36	64				
P7	Calhetas	22	13	33.2	37	12		1		1
P8	Segredo	25	9	28.2	35.5	16		33		

To identify and characterize prey availability six quadrats of 100 cm^2 of tide pool surface were randomly scrapped to bare rock, on the inside of each tide pool. In the intertidal zone adjacent to each tide pool six quadrats were also scrapped, that is, in the intertidal area that is discovered at low tide just outside the pool (the six quadrats were placed in the rocky platform all around each pool, in close proximity to the pool at ~20 cm from the pool's edge). The same sampling of six quadrats was performed in adjacent subtidal habitats (the closest subtidal area in a seaward direction was chosen for standardization purposes, samples were taken at a depth of approximately 30 cm). The quadrats were scrapped with a flat chisel with 5 cm on its scrapping edge. This was done by hand and the scrapped material was pushed into a plastic bag very carefully to minimize losses. All potential prey found within each quadrats were collected, preserved in 70% alcohol and transported to the laboratory, where were identified with a stereomicroscope, measured (Lt), precision of 0.03 mm and weighed (Wt), precision of 0.0001 g.

Transient fish present in pools were collected with hand nets (1 mm mesh), transported to the laboratory in refrigerated bags and preserved at −20 °C. Fish identification was confirmed, and these were measured (total length with a precision of 1 mm) and weighed (wet weight with a precision of 0.01 g). These are fish that were observed in the pools but are not resident. They stays in the pools during ebb tide but disperse in the intertidal when the tide comes in.

Stomachs were excised and contents were removed for identification. The stomach contents of four transient fish species were analysed. From the family Pomacentridae 126 stomachs of the species *Abudefduf saxatilis* ranging from 8 to 56 mm were analysed, from the family Sparidae 37 stomachs of the species *Diplodus argenteus*, ranging from 9 to 40 mm, were analysed, from the family Gerreidae 96 stomachs of juveniles, ranging from 10 to 46 mm and 179 stomachs of post-larvae, ranging from 7 to 17 mm, of the species *Eucinostomus melanopterus* were analysed. *E. melanopterus* were classified as post-larvae or juvenile, based on their body shape, which is elongated for post-larvae and more triangular for juveniles. Each prey item was identified at the lowest taxonomic level possible, counted and weighed.

The sampling followed the Brazilian legislation and ethics committees in Brazil specifically authorized this work (authorization document 13.1.981.53.7 from the Brazilian authorities (CEUA, USP—Ribeirão Preto)). The field work did not involve endangered or protected species.

6.2.3. Diet characterization

To estimate the importance of each prey item in the diet of *A. saxatilis*, *D. argenteus*, *E. melanopterus* and *E. melanopterus* post-larvae three dietary indices were determined (e.g. Hyslop, 1980). The numerical index (NI) represents the percentage of the number of individuals of a prey over the total number of individuals of all prey. The occurrence index (OI) represents percentage of non-empty stomachs in which a prey occurred over the total number of occurrences. The gravimetric index (GI) consists of the percentage by wet weight of a prey on the total weight of all prey items (e.g. Hyslop, 1980).

To evaluate the degree of overlap in the diet between species we used the Schoener (1968) index T, formula 6.1:

$$T = 1 - 0.5\Sigma \left| px_i - py_i \right|$$

where px_i and py_i are the estimated proportions by weight of prey "*i*" in the diets of species x and y, respectively. This measure ranges from 0 (species use totally different resources) to 1 (all prey items are found in equal proportions). Diet overlap was considered low if range 0.0–0.39, intermediate if range 0.40–0.60 and high if greater than 0.60 (Langton, 1982).

6.2.4. Prey-predator interactions

The electivity index, according to Strauss (1979), was used to describe prey selection by species in this study and is represented by the expression $L = r_i - p_i$, where r_i is the relative frequency of the item *i* in the diet, and p_i is the relative frequency of the item *i* in the environment. This index compares the proportion of different prey categories to the diet with its proportion in the environment. It ranges from −1 (inaccessibility of the prey item) to 1 (prefect selection for a prey item), zero indicates random selection from the environment. In this analysis the *E. melanopterus* post-larvae individuals were not considered.

6.2.5. Individual variation

We used the proposed adaptation of PS to measure individual level diet variation (PS_i), which measures the overlap between an individual's diet and that of the population, because Bolnick et al. (2002) noted that this method could be applied to any axis of niche. The most specialized individuals, those with the lowest index, have diets that are the most distinct from the rest of the population, while individuals that consume prey in direct proportion to the population as a whole have PS_i equal 1. The average of the PS_i values of all individuals in the population can be summarized as a population-wide measure of individual specialization (IS) (Bolnick et al., 2002; Araújo et al., 2007). IS varies from near 0 (maximum individual specialization) to 1 (no individual specialization). We calculate this index used the program IndSpec 1 provided by the authors (Bolnick et al., 2002). Details about index calculations are described in the instruction of the IndSpec 1 program. The significance tests ANOVA with Tukey post-hoc tests to test for differences between fish species were performed with STATISTICA 12. The P-values < 0.05 was considered significant. In this analysis the *E. melanopterus* post-larvae individuals were not considered.

6.2.6. Data analysis

Statistical analyses were conducted with the software PRIMER (Clarke & Warwick, 2007). For each tide pool, permutational multivariate analysis of variance (PERMANOVA) was used to analyze if there differences in potential prey at the three sampled zones (inside the pools, outside the pools and in the nearest adjacent subtidal zone). The analysis of similarity percentages (SIMPER) was used to calculate the contribution of each species (%) to the dissimilarity between each zones.

To evaluate the importance of the intertidal tide pools in the feeding ecology of the most abundant transient fish species, we compared the stomach contents matrices with the matrices of the potential prey identified in the different sampled zones of the intertidal (inside, outside and subtidal zone). This association was evaluated through the linking biotic patterns to environmental variables (BEST analysis). For this BEST analysis, the pools with the largest number of individuals were selected for each species, ie, for the *A. saxatilis* species, pools 2, 3, 6, 7 and 8 were selected, for the *D. argenteus* species pools 3 and 4 and for *E. melanopterus* pools 4 and 8 were selected. In this analysis we considered high correlation (R^2) when the mean values were higher than 0.6. All analyses were performed on square-root transformed data using the Bray-Curtis similarity matrix.

6.3. Results

6.3.1. Distribution and abundance

A total of 4 species of non-resident fish were identified in the pools: *Abudefduf saxatilis* (Linnaeus, 1758) (n = 126, Lt 30.37 ± 11.00 mm, Wt 0.76 ± 0.70 g), *Diplodus argenteus* (Valenciennes, 1830) (n = 37, Lt 18.78 ± 6.25 mm, Wt 0.17 ± 0.16 g), *Eucinostomus melanopterus* (Bleeker, 1863) (juvenile, n = 96, Lt 31.28 ± 6.63 mm, Wt 0.44 ± 0.22 g; post-larvae, n = 179, Lt 11.15 ± 1.66 mm, Wt 0.001 ± 0.00 g) and *Odontesthes argentinensis* (Valenciennes, 1835) (n = 6, Lt 32.50 ± 3.89 mm, Wt 0.38 ± 0.14 g). The species *O. argentinensis* was considered a rare species because its occurrence in the intertidal tide pools was very low.

6.3.2. Prey availability

A total of 430 prey available representing 52 taxa were sampled inside the pools (Table 6.2). These potential feeding areas were dominated by copepods, represented by 2 families (Harpacticidae and Metidae); polychaetes, represented by 10 families, the most abundant being Capitellidae, Paraonidae and Cirratulidae; amphipods, represented by 9 families, the most abundant being Amphitoidae, Corophiidae and Gammaridae; and Tanaidacea, represented by 2 families, Kalliapseudidae and Leptocheliidae (Table 6.2).

A total of 187 prey available representing 25 taxa were sampled in intertidal zone adjacent to each tide pool (Table 6.2). The most abundant taxa were copepods, bivalves, maxillopoda and gastropods. Poriferans and the barnacle species *Tetraclita stalactifera* occurred only in this area (Table 6.2).

In the subtidal zone, a total of 625 prey available representing 46 taxa were sampled (Table 6.2). The taxa Tanaidacea, Amphipoda, Polychaeta and Copepoda were the most abundant. The Platlyhelminthes, Mysidae and the species *Protopalythoa variabilis* occurred only in this area.

The three zones defined for the assessment of prey availability were significantly different in all sampled tide pools, with high values of dissimilarity (Table 6.3).

Table 6.2. Relative frequency of main taxa in the different sampled habitats (tide pools, intertidal zone and adjacent subtidal habitats).

Prey available		Relative frequency Inside tide pools	Intertidal zone	Subtidal zone
Chlorophyta	∑	0.037	0.075	0.022
Ulva sp.		0.019	0.037	0.011
Rhizoclonium sp.		0.019	0.037	0.011
Phaeophyceae	∑	0.007	0.000	0.002
Sargassum cymosum		0.002	0.000	0.000
Bachelotia sp.		0.000	0.000	0.002
Sphacelaria sp.		0.000	0.000	0.000
Padina sp.		0.002	0.000	0.000
Dictyopteris sp.		0.002	0.000	0.000
Rhodophyta	∑	0.035	0.011	0.029
Asparagopsis sp.		0.000	0.000	0.002
Acanthopora spicifera		0.007	0.000	0.000
Ceramium sp.		0.007	0.005	0.011
Bostrychia sp.		0.000	0.000	0.002
Hypnea musciformis		0.014	0.005	0.010
Jania rubens		0.002	0.000	0.000
Amphiroa sp.		0.005	0.000	0.005
Porifera	∑	0.000	0.005	0.000
Anthozoa	∑	0.000	0.000	0.008
Protopalythoa variabilis		0.000	0.000	0.008
Platlyhelminthes	∑	0.000	0.000	0.003
Nematoda	∑	0.016	0.027	0.010
Nemertea	∑	0.002	0.000	0.003
Polychaeta	∑	0.188	0.075	0.142
Amphinomidae		0.000	0.000	0.000
Capitellidae		0.049	0.032	0.000
Cirratulidae		0.028	0.000	0.034
Magelonidae		0.009	0.000	0.000
Maldonidae		0.016	0.000	0.005
Nephtyidae		0.016	0.000	0.018
Nereididae		0.014	0.021	0.077
Opheliidae		0.000	0.011	0.000
Paraonidae		0.033	0.011	0.005
Phyllodocidae		0.000	0.000	0.005
Pilargidae		0.016	0.000	0.000
Polygordiidae		0.002	0.000	0.000
Terebellidae		0.005	0.000	0.000
Gastropoda	∑	0.033	0.107	0.010
Cerithium atratum		0.007	0.000	0.000
Fissurella clenchi		0.000	0.000	0.005
Littorina flava		0.000	0.011	0.000
Morula nodulosa		0.002	0.000	0.005
Nodilittorina lineolata		0.000	0.096	0.000
Petaloconchus sp.		0.009	0.000	0.000
Stramonita haemastoma		0.007	0.000	0.000
Turbonilla abrupta		0.005	0.000	0.000
Lottia subrugosa		0.002	0.000	0.000
Bivalvia	∑	0.035	0.144	0.014
Donax sp.		0.002	0.000	0.000
Perna perna		0.028	0.123	0.010

Sphenia fragilis		0.005	0.021	0.005
Ostracoda	∑	0.005	0.000	0.003
Mysidae	∑	0.000	0.000	0.003
Tanaidacea	∑	0.065	0.070	0.278
Kalliapseudidae		0.056	0.059	0.181
Leptocheliidae		0.009	0.011	0.098
Isopoda	∑	0.007	0.027	0.011
Cirolanidae		0.000	0.000	0.003
Sphaeromatidae		0.007	0.027	0.008
Amphipoda	∑	0.184	0.037	0.242
Caprellidae		0.002	0.000	0.000
Ampithoidae		0.058	0.000	0.058
Colomastigidae		0.002	0.000	0.005
Corophiidae		0.030	0.032	0.067
Gammaridae		0.028	0.005	0.074
Lysianassidae		0.014	0.000	0.008
Melitidae		0.016	0.000	0.005
Stenothoidae		0.000	0.000	0.010
Talitridae		0.026	0.000	0.010
Hyalidae		0.000	0.000	0.006
Aoridae		0.007	0.000	0.000
Decapoda	∑	0.040	0.000	0.021
Epialtus brasiliensis		0.005	0.000	0.000
Pagurus sp.		0.026	0.000	0.006
Panopeus sp.		0.009	0.000	0.010
Pilumnus reticulatus		0.000	0.000	0.005
Maxillopoda	∑		0.128	0.000
Chthamalus sp.		0.000	0.128	0.000
Copepoda	∑	0.305	0.257	0.122
Harpacticoida		0.233	0.144	0.109
Metidae		0.072	0.112	0.013
Arachnida	∑	0.007	0.016	0.010
Arachnida n.i.		0.005	0.016	0.000
Hydrachnidia		0.002	0.000	0.010
Insecta	∑	0.019	0.021	0.038
Insecta n.i.		0.005	0.000	0.000
Chironomidae		0.014	0.021	0.038
Ophiuroidea	∑	0.016	0.000	0.029
Ophiuroidea n.i.		0.007	0.000	0.000
Amphiuridae		0.009	0.000	0.014
Ophiactidae		0.000	0.000	0.014

Table 6.3. Results of the PERMANOVA and SIMPER analysis testing differences in potential prey at the three sampled zones (inside the pools, outside the pools and in the adjacent subtidal zone) for each tide pool.

PERMANOVA							
Location	Source	Degrees of fredoom	Sum of squares	Average of squares	Pseudo-F	P (perm)	Unique perms
P1	Zo = Zone	2	7174.3	3587.1	3.7125	0.003	999
	Residual	15	14493	966.22			
	Total	17	21668				
P2	Zo = Zone	2	14855	7427.7	3.0611	0.006	997
	Residual	15	36397	2426.4			
	Total	17	51252				
P3	Zo = Zone	2	26497	13248	6.3862	0.001	998
	Residual	15	31118	2074.6			
	Total	17	57615				
P4	Zo = Zone	2	12231	6115.3	2.461	0.024	999
	Residual	15	37272	2484.8			
	Total	17	49503				
P5	Zo = Zone	2	25117	12558	8.989	0.001	999
	Residual	15	20956	1397.1			
	Total	17	46073				
P6	Zo = Zone	2	34636	17318	18.843	0.001	995
	Residual	15	13786	919.06			
	Total	17	48422				
P7	Zo = Zone	2	37965	18983	20.409	0.001	992
	Residual	15	13952	930.12			
	Total	17	51917				
P8	Zo = Zone	2	12971	6485.3	2.9741	0.003	997
	Residual	15	32708	2180.6			
	Total	17	45679				

SIMPER - Average dissimilarity (%)								
Pool	1	2	3	4	5	6	7	8
Inside vs Outside	55.76	85.95	94.35	80.34	65.93	90.73	100	77.15
Inside vs Subtidal	38.76	57.69	73.29	63.18	78.57	62.36	58	62.63
Outside vs Subtidal	54.78	88.34	94.29	82.61	90.54	98.06	100	78.82

6.3.3. Feeding ecology

The diet of *A. saxatilis* encompassed 25 different prey items (Table 6.4). The most frequent prey group was Crustacea of all prey found in the stomachs. The most important prey item within the Crustacea group were copepods and amphipods. Brown algae (Phaeophyceae) and green algae (Chlorophyta) are also common and important resource, according to the three indices. Gravimetrically, algae are more important resources than the Crustacea group. The sand also has some importance in the gravimetric index, being ingested during the feeding process.

D. argenteus diet was also composed mainly of Crustacea, dominated by harpacticoid copepods (Table 6.4). Insects and green algae were also important food items according to the three indices.

The juvenile *E. melanopterus* diet was composed of 17 prey items (Table 6.4), the most frequent prey group was Crustacea, dominated by copepods, Harpacticoida and Metidae. Metidae was the second most important food item in terms of numerical and gravimetric indices and the third in terms of occurrence index. Green algae were important resource according to the numerical and gravimetric indices but more important than Metidae in relation to the occurrence index. The Polychaeta group are low index numerically values but is very important in relation to the gravimetric and occurrence indices. Gravimetrically, *Eucinostomus sp*. fish larvae are also an important food item.

The diet of *E. melanopterus* post-larvae was composed of 3 prey items (Table 6.4). The most important resource was green algae of all prey found in the stomachs. Porifera was the second most important food item in terms of numerical index and the third in relation to the volumetric and occurrence indices. Phytoplankton was the third most important group according to the numerical index but the second in terms of the volumetric and occurrence indices.

Table 6.4. Numerical, occurrence and gravimetric index values of prey found in stomachs of *Abudefduf saxatilis*, *Diplodus argenteus* and *Eucinomostomus melanopterus* (juvenile and post-larvae).

		Abudefduf saxatilis			***Diplodus argenteus***			***Eucinostomus melanopterus***			***Eucinostomus melanopterus* post-larvae**		
Food items		**IN**	**IG**	**IO**	**IN**	**IG**	**IO**	**IN**	**IG**	**IO**	**IN**	**IG**	**IO**
Phytoplankton	∑	0.00	0.00	0.00	0.19	1.17	2.41	0.05	0.04	0.91	14.89	9.37	15.45
Chlorophyta	∑	2.16	36.45	23.03	6.04	16.63	18.07	0.89	4.39	16.82	69.05	37.24	61.37
Ulva sp.		0.05	0.41	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Enteromorpha sp.		0.05	0.13	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chlorophyta n.i.		2.05	35.91	21.91	6.04	16.63	18.07	0.89	4.39	16.82	69.05	37.24	61.37
Phaeophyceae	∑	2.15	32.11	23.03	0.00	0.00	0.00	0.10	0.49	1.82	0.00	0.00	0.00
Sphacelaria sp.		0.58	8.85	6.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hypnea musciformis		0.53	7.89	5.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Padina gymnospora		0.05	0.65	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acanthophora spicifera		0.03	0.04	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phaeophyceae n.i.		0.97	14.68	10.39	0.00	0.00	0.00	0.10	0.49	1.82	0.00	0.00	0.00
Rhodophyta	∑	0.29	0.83	3.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ceramium sp.		0.29	0.83	3.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Porifera	∑	0.16	0.50	1.69	0.66	7.46	8.43	0.05	0.45	0.91	16.06	8.59	14.16
Nematoda	∑	0.03	0.04	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Annelida	∑	0.35	1.10	3.09	0.00	0.00	0.00	0.72	7.48	9.54	0.00	0.00	0.00
Polychaeta n.i.		0.24	0.71	2.25	0.00	0.00	0.00	0.65	6.53	8.18	0.00	0.00	0.00
Amphinomidae		0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.59	0.45	0.00	0.00	0.00
Opheliidae		0.11	0.39	0.84	0.00	0.00	0.00	0.05	0.36	0.91	0.00	0.00	0.00
Mollusca	∑	0.03	0.07	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gastropoda	∑	0.03	0.07	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Arthropoda	∑	94.69	17.20	42.98	93.12	74.73	71.08	97.94	82.83	66.35	0.00	0.00	0.00
Crustacea	∑	94.08	16.42	39.61	86.42	56.61	50.60	97.56	81.95	60.89	0.00	0.00	0.00
Tanaidacea		0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.20	0.91	0.00	0.00	0.00
Mysidacea		0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.45	0.00	0.00	0.00
Isopoda		0.21	0.48	1.69	0.38	4.16	4.82	0.00	0.00	0.00	0.00	0.00	0.00
Amphipoda	∑	2.89	4.66	4.78	0.95	11.09	12.05	0.45	1.73	4.54	0.00	0.00	0.00
Gammaridae		0.42	2.37	3.09	0.57	5.97	7.23	0.43	1.37	4.09	0.00	0.00	0.00
Caprellidae		0.00	0.00	0.00	0.38	5.12	4.82	0.02	0.36	0.45	0.00	0.00	0.00
Amphipoda n.i		2.47	2.29	1.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Copepoda	∑	90.90	11.26	32.58	85.09	41.36	33.73	93.38	76.29	50.90	0.00	0.00	0.00
Harpacticoida		90.88	11.25	32.30	85.09	41.36	33.73	59.81	49.28	40.45	0.00	0.00	0.00
Metidae		0.03	0.01	0.28	0.00	0.00	0.00	33.57	27.01	10.45	0.00	0.00	0.00
Cypris		0.08	0.02	0.56	0.00	0.00	0.00	3.66	3.71	4.09	0.00	0.00	0.00
Insecta	∑	0.32	0.69	2.81	6.42	16.84	18.07	0.12	0.50	1.82	0.00	0.00	0.00
Chironomidae		0.29	0.62	2.53	6.23	14.07	15.66	0.12	0.50	1.82	0.00	0.00	0.00
Insecta n.i.		0.03	0.07	0.28	0.19	2.77	2.41	0.00	0.00	0.00	0.00	0.00	0.00
Acari	∑	0.29	0.09	0.56	0.28	1.28	2.41	0.26	0.38	3.64	0.00	0.00	0.00
Hydrachnidia		0.29	0.09	0.56	0.28	1.28	2.41	0.26	0.38	3.64	0.00	0.00	0.00
Teleostei	∑	0.00	0.00	0.00	0.00	0.00	0.00	0.24	4.34	1.82	0.00	0.00	0.00
Eucinosmotus sp.		0.00	0.00	0.00	0.00	0.00	0.00	0.24	4.34	1.82	0.00	0.00	0.00

Sand	∑	0.13	11.49	1.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rocks	∑	0.03	0.18	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Percentage of empty stomachs			0.84			0.00			1.82			9.01	

6.3.4. Niche overlap

All Schoener (1968) indices of dietary overlap were higher than 0.60, an indicator of high dietary overlap (Langton, 1982). The diet overlap was higher between *D. argenteus* and *A. saxatilis* (T = 0.90) and lower between *E. melanopterus* and *A. saxatilis* (T = 0.63) (Table 6.5).

Table 6.5. Schoeners index (SI) of diet niche overlap.

	Abudefduf saxatilis	*Eucinostomus melanopterus*	*Diplodus argenteus*
Eucinostomus melanopterus	0.63		
Diplodus argenteus	0.90	0.75	
Eucinostomus melanopterus post-larvae	0.68	0.78	0.76

6.3.5. Electivity index (S)

According to the electivity index (S), the species exhibited a similar prey selection pattern (Fig. 6.2). For *A. saxatilis*, positive index values were obtained for the three most important prey item (harpacticoid copepods, green algae and brown algae) and negative for amphipods, in the three areas sampled (inside, outside and subtidal).

The index showed that *D. argenteus* positively selected the three most abundant items of the diet (harpacticoid copepods, green algae and insects), in the three areas. For juvenile *E. melanopterus* the index was strongly positive for all areas for the prey item harpacticoid copepods, green algae and brow algae but the prey item Metidae were negatively selected at inside and subtidal areas and positive in the outside area.

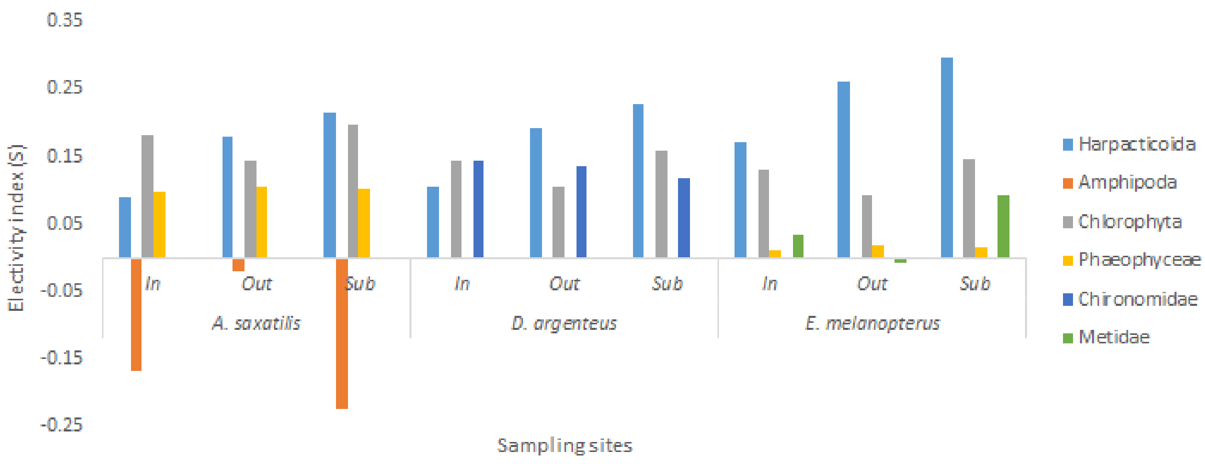

Figure 6.2. Electivity values for the main prey items of *A. saxatilis*, *D. argenteus* and *E. melanopterus* in sampling areas (inside tide pools, outside and subtidal habitats).

6.3.6. Individual variation

High PS_i values showed that the three fish species do not have individual specialization (Fig. 6.3). Comparison of the PS_i values between the three species, shows that the PS_i values of *A. saxatilis* were different from the others but they were not different between *D. argenteus* and juvenile *E. melanopterus*. Individual specialization (IS) was weaker (higher: IS = 0.76) in *A. saxatilis*. The IS for *D. argenteus* and juvenile *E. melanopterus* was 0.64 and 0.59, respectively.

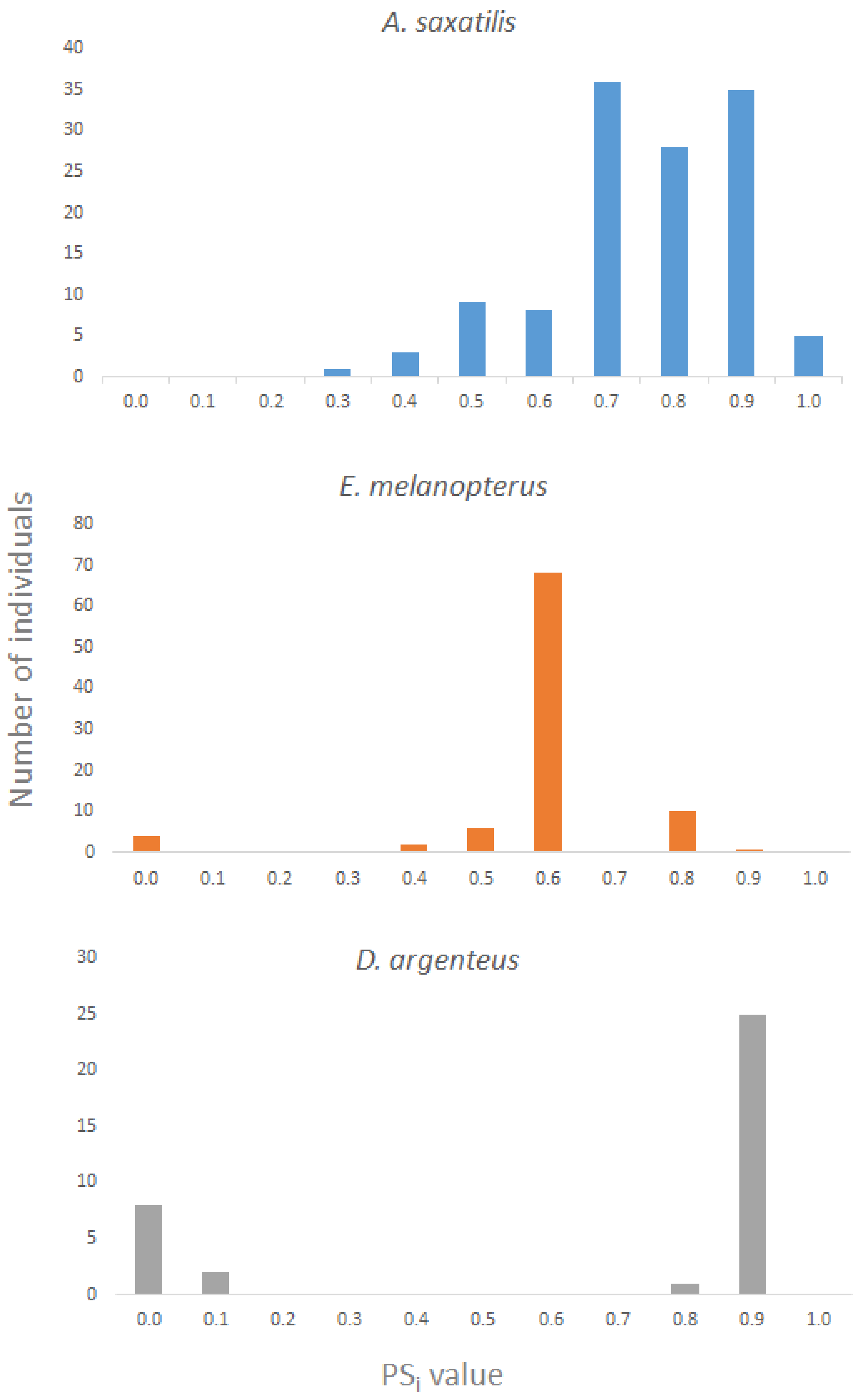

Figure 6.3. Individual specialization of *A. saxatilis*, *D. argenteus* and *E. melanopterus*.

6.3.7. Association between stomach contents and prey available in the three areas (inside the pool, outside the pool and in the subtidal)

Correlational evidence was obtained from different tide pools depending on the distribution and abundance of fish species (n = 5 for *A. saxatilis*; n = 2 for *E. melanopterus*; n = 2 for *D. argenteus*). In total, for *A. saxatilis* 112 individuals were examined for *E. melanopterus* 91 and for *D. argenteus* 37 individuals.

For *A. saxatilis*, the BEST analysis showed that there is a higher mean correlation between ingested food items and the species identified within the tide pool and the subtidal zone (R^2 = 0.72 and R^2 = 0.69, respectively) (Table 6.6). The diet of the species *E. melanopterus* presents a intermediate average correlation with the species that occur in the intertidal habitat outside the pool and inside tide pools (R^2 = 0.53 and R^2 = 0.52, respectively) (Table 6.6). The diet of *D. argenteus* was best correlated to the presence of food items inside the pools (R^2 = 0.83) (Table 6.6).

Table 6.6. BEST analysis testing the correlation between stomach contents and food availability at the three sampled habitats (tide pools, nearby intertidal and adjacent subtidal habitats).

BEST		
Zone	**Number of variable**	**Correlation**
A.saxatilis		
Inside pool	3	0.72
Outside pool	1	0.44
Subtidal	4	0.69
E. melanopterus		
Inside pool	2.5	0.52
Outside pool	4	0.53
Subtidal	2.5	0.3
D. argenteus		
Inside pool	3	0.83
Outside pool	3	0.44
Subtidal	2	0.37

6.4. Discussion

This study indicates that intertidal rock pools may be broadly used as feeding grounds by juvenile transient fish; a function that might parallel the importance of tide pools as a habitat providing refuge from predators or a metabolic advantage during early juvenile development (White et al., 2015). This had been put forward by several authors, but there were no prior attempts to test this hypothesis using correlational evidence from stomach contents and potential foraging habitats.

The importance of tide pools apparently relies on the fact that all species examined extensively preyed on copepods, which were abundant on the tide pool environment. In fact, most larval and post-larval stages of fish species feed mainly on copepods probably due to their abundance and accessibility, as well as their small size and easy assimilation, (Baldo & Drake, 2002; Elliott et al., 2002; Evjemo et al., 2003; Gning et al., 2010). According to Evjemo et al. (2003), copepods have very high protein contents which are essential for growth and survival of fish during their early life stages. Copepods were the most consumed food item and their generalized consumption explained the high niche overlap found between the three species. The dominance of any given food item may be related not only to its selectivity, but also to its abundance and availability (Klumpp & Nichols, 1983). In tide pools, the correlational evidence reported in this study suggests that the copepod dominance in fish stomachs owes to the fact that copepods are very abundant on the benthic habitats foraged by these juvenile fish.

The species *Abudefduf saxatilis* and *Diplodus argenteus* have previously been described as frequent in Brazilian intertidal rock pools (Barreiros et al., 2004). There is marked diet overlap between these two species, but interspecific competition may be reduced because juvenile recruitment takes place in summer in *A. saxatilis* and in winter in *D. argenteus* (Allen, 1991; Cervigón, 1993). Notwithstanding, we found juveniles of both species coexisting in pools during summer, indicating that at this latitude their recruitment seasons may overlap.

The species *Eucinomostomus melanopterus* was the most abundant fish species at the sampled tide pools, followed by *Abudefduf saxatilis*, *Diplodus argenteus* and *Odontesthes argentinensis*. The Pomacentridae fish *A. saxatilis* is one of the most common species in Brazilian rocky reefs and has its peak abundance in summer (Adelir-Alves et al., 2016), so the high abundance registered in this study was not surprising. It is not clear, however, if summer fish recruits home to nursery tide pools or whether higher density in pools is maintained by frequent immigration of nearshore juveniles fishes that use pools as nursery grounds (Amara & Paul, 2003). Copepods and amphipods were the most important prey items in the diet of *A. saxatilis,* followed by Phaeophyceae and Chlorophyta algae, supporting the idea that species of the Pomacentridae family are opportunistic omnivores (Frederich et al., 2009). The diet composition reported in this study agrees with results presented by Adelir-Alves et al. (2016) for sergeant major fish in subtidal waters within the same region. Since the diet of intertidal *A. saxatilis* fish was similarly correlated to the tide pool and adjacent subtidal habitat, we conclude that preferred feeding sources, namely green algae and harpacticoid copepods, are supplied in both habitats, and that tide pools may be viewed as an extension of potential nursery grounds where fish may also benefit from other ecological functions.

The silver porgy *Diplodus argenteus* is a generalist omnivorous species, feeding on algae and invertebrates (Carpenter & Russell, 2014). In this study, we found that copepods were the most important prey item in its diet, followed by insects and green alga, which roughly corresponds to the diet of this same species in southern Brazil (Dubiaski-Silva & Masunari, 2004), and to the diets of the congeners *D. holbrooki* (Pike & Lindquist, 1994), *D. sargus capensis* (Coetzee, 1986) and *D. vulgaris* (Gonçalves & Erzini, 1998). The tight correlation observed between the diet composition of *D. argenteus* and the abundance of feeding items in the pool habitat indicates that tide pools are potentially important for this species. The number of this fish species in tide pools was however low, and inferior to the estimates observed by others (Barreiros et al., 2004), probably because this study was carried out in summer, not in winter, when juvenile recruitment of *D. argenteus* peaks (Barreiros et al., 2004).

The species *E. melanopterus* was frequent in our study. It is a euryhaline species with a wide environmental tolerance (Gning et al., 2010), and typical opportunistic feeding habit, probably seeking shelter and/or food to and from estuarine nursery grounds, similarly to the patterns described at intertidal pools of rocky reefs in Iparana, Ceará (Brazil) (Cunha et al., 2008). The most important prey item in this fish species diet was copepods, green and brown algae. These data are in agreement with the results obtained by other authors who investigated the diet of this species in estuaries, where it was observed that the main food item for both larvae and post-larvae is copepods (Fagade & Olaniyan, 1973; Gning et al., 2008, 2010). There was a greater correlation between the diet of *E. melanoterus* and the occurrence of potential prey in intertidal habitats within and outside pools. The use of intertidal out-of-pool resources is not surprising since it has strong ability to exploit different environmental niches (Cunha et al., 2007).

In general, the three fish species studied positively elected the same prey items inside the pools, outside the pools and in the adjacent subtidal area, which means that they do not stay in the pools to feed on any particular item. It was also observed that there was no individual specialization, with most individuals of the three species consuming prey in the same proportions as the population as a whole. However, correlational evidence (given by the BEST analysis) showed similarities between stomach content composition and prey availability inside the pools.

This study is the first to investigate the association between the stomach content of intertidal rock pool fishes and the food availability of the surrounding habitats. Our results show a consistent similarity between stomach contents and the occurrence of potential prey items inside of tide pools, for all species investigated, indicating that intertidal rock pools are likely important feeding grounds for transient juvenile marine fish.

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CHAPTER 7

Final remarks and future perspectives

7.1. Final remarks

Today's natural ecosystems are changing and facing a massive decline in biodiversity with thus far unknown consequences. Therefore, an understanding of the complexity inherent to ecosystem functioning is crucial for the preparation for future scenarios of global change. This thesis aims to contribute to the understanding of the complexity and the maintenance of diversity in ecosystems based on the study of food web network structure. This work showed that intertidal rock pools provide an excellent model for the study of marine food web networks. This conclusion was reached on the basis of a large-scale sampling effort covering 116 intertidal rock pools, located at different latitudes and ecoregions of the world: Celtic Sea (United Kingdom, 50ºN), Gulf of Saint Lawrence (Canada, 48ºN), South European Atlantic shelf (Portugal, 38ºN), Madeira Island (Portugal, 32ºN), Northeast Brazil (Brazil, 3ºS), Southeast Brazil (Brazil, 23ºS), and can, thus, be considered a robust finding that opens new avenues for future research.

Several other research questions were proposed in chapter 1 and will now be answered in the context of the results obtained:

Which are the most vulnerable food webs to species loss, temperate or tropical?

In recent years, several studies concluded that tropical ecosystems are more vulnerable and will probably lose more species in the future, than temperate ecosystems. This work showed, for the first time, that tropical food web networks are generally more robust to species loss than temperate food webs, in classical species deletions simulations. This means that, although tropical ecosystems will probably lose more species, their food web networks seem to be more robust to species' loss than temperate webs. Interestingly, tropical ecosystems seem to be more vulnerable at the species level, but going up in the biological organization scale, this work showed that they may be more robust at the food web network level than temperate ecosystems. This conclusion was taken using various theoretical extinction sequences (e.g. from the most connected to the least connected species), that are unlikely to occur in natural ecosystems. Nevertheless, this is a relevant exercise that allows the comparison of the webs studied here with others, since the extinction sequences tested here are the most commonly used in food web network theory.

In this context, this work confirmed that the removal of highly connected species is particularly disruptive for food webs, while showing differences in robustness among temperate and tropical food webs, for the first time in this field.

The fact that tropical webs, albeit more robust, generally suffered more alterations in its properties due to species loss, than temperate webs, is quite relevant since it may incur alterations in ecosystem functions.

What are the species' traits associated with secondary extinction risk?

The temperate and tropical food webs were less robust when the removal was directed at the most-connected species, confirming that highly connected species are key-species in food webs. The tropical webs presented a robustness to the removal of species directed at the "most-connected" species of 32% and the temperate webs a robustness of only 27%. Temperate webs presented higher proportions of intermediate, herbivorous and cannibalistic species, and removals affected intermediate species' proportion more than the other trophic groups, in the "most-connected" exercise, this way, it is hypothesized that this high proportion of intermediate, highly connected species in the web, may result in less robust webs to the removal of highly connected species. The higher robustness of tropical webs can be related to a number of aspects of the network topology prior to removals, such as highest proportion of top, basal and omnivorous species. This relation and its reasons are uncertain and should be further investigated in future studies.

What are the consequences for the structure of food webs, when species vulnerable to warming are removed?

Temperate and tropical food web networks presented similar structural robustness when species were sequentially removed based on their thermal vulnerability. This is a very interesting result, given that on a set of classical theoretical removal sequences tropical webs had been generally more robust to species loss. This shows just how important it is to use extinction sequences that are based on realistic criteria. The thermal vulnerability criteria used here is very relevant and timely, given today's concerns over climate warming, however other vulnerability rankings should be produced to be used in food web ecology, such as vulnerability to oil contamination, common contaminant mixtures, to ocean acidification and to other common and emerging threats to biological systems.

Are tropical food webs more vulnerable to warming than temperate food webs?

Tropical food web networks are not more susceptible to warming in structural terms, but the heatwave exercise conducted in this work showed that the tropical webs lose a much higher number of species and present much more alterations in network properties. This happens because on a realistic scenario, a much higher number of species have a critical thermal maximum below the maximum habitat temperature and were thus removed. So tropical food webs are much more likely to suffer network alterations because they encompass a much higher number of vulnerable species. Such a loss will likely result is food web network structure alterations. This adds to the body of evidence supporting the relative higher vulnerability of tropical ecosystems to warming, showing the need to prioritize research, conservation and environmental management actions in tropical ecosystems.

Do food web properties, estimated from intertidal rock pool biodiversity samples, vary seasonally in temperate regions?

No seasonal variation was detected in the proportion of top, intermediate and basal species, as well as on the proportion of herbivores, omnivores and cannibal species. This means that overall the constitution of these temperate food web networks is quite stable and keeps its basic topology throughout the year. However, the food web networks encompassed more taxa in summer and autumn than in spring and winter, mostly due to an increment in macroalgal species and transient marine fishes. Connectance was lower in summer and autumn which may mean lower network robustness. Mean shortest path length was higher in spring and summer, which may counterbalance the lower connectivity in summer. Although with little associated seasonality, anemones and resident fish were important top predators in the webs, revealing their important role in these food webs. Some rare species, like the octopus, always occupy top predator level whenever present in these webs. These are highly valuable commercial species and are targeted by local fisheries in pools.

Do marine fish juveniles use intertidal rock pools as feeding grounds?

This was the only work in this thesis not directly involving food web networks analysis. However, the information gathered in this work is important for the establishment and confirmation of feeding links assumed to exist in the food web networks produced for this work. If a species is present in a habitat, it is generally assumed that it is part of the local food web. Early-stages of transient fish occur in intertidal rock pools in many parts of the world, and it is assumed that one of the benefits they take from these pools is abundant food, however their trophic role in the pools had never been confirmed. This study showed, for the first time, that the juveniles of the three fish species studied, *Abudefduf saxatilis* (Linnaeus, 1758), *Diplodus argenteus* (Valenciennes, 1830), *Eucinostomus melanopterus* (Bleeker, 1863), positively elect the prey items inside the pools. Results showed consistently higher similarity between stomach contents and the occurrence of potential prey items inside of tide pools *versus* the alternative feeding habitats, for all species investigated, thus confirming these species as part of the pool's food webs.

In summary, the thesis' main conclusions are listed:

- ✓ The food webs from intertidal rock pools have a great potential to be used as proxies of larger marine ecosystems for food web networks research;
- ✓ The tropical food web networks are generally more robust to species loss than temperate food webs, in classical theoretical removal exercises;
- ✓ The number of network properties affected was higher in tropical webs, in classical theoretical removal exercises, indicating that their structure was more deeply affected by species loss;
- ✓ Highly connected species are key species in food webs. This was confirmed, once the temperate and tropical food webs were less robust when the removal was directed at the most-connected species;
- ✓ The positive logarithmic relation previously found between robustness and connectance was only confirmed for temperate webs, highlighting the importance of including tropical case-studies in datasets;
- ✓ Temperate and tropical food web networks presented similar structural robustness when species were sequentially removed based on their thermal vulnerability;

- ✓ Tropical food webs are more vulnerable to warming than temperate food webs on a realistic warming scenario, not because their networks are less robust but because they encompass a much higher number of thermally vulnerable species and, consequently will present much more alterations in network properties due to their loss;
- ✓ No seasonal variation was detected in the food web networks of intertidal rock pools;
- ✓ Anemones and resident fish are important predators in the webs, but with little seasonality associated;
- ✓ Intertidal rock pools are likely important feeding grounds for transient juvenile marine fish.

7.2. Future perspectives

Intertidal rock pools are small and easy to sample, allowing high replication and easy manipulation, two of the main challenges when dealing with large open systems. The present study was the first showing the great potential of intertidal rock pools as proxies for larger marine ecosystems, for food web networks research. Although there are surely some limitations (e.g. absence of large size organisms) inherent to the use of this environment as proxy, the use of intertidal rock pools for food web networks research will allow important advances in the understanding of the complex organization of ecosystems. Its' contained structure allows a precise delineation and manipulation of communities. This way, future studies can use them as natural laboratories for experimental manipulation of the web components and abiotic variables (e.g. algal coverage manipulation, predators' exclusion, temperature, salinity).

From a methodological perspective, efforts should be done to deepen the existing knowledge on food webs networks in every habitat type, all over the world. Theoretical and computational models need to be tested against more diverse, more complete, and more highly resolved data. It is important to make a sampling effort to obtain detailed and evenly resolved data of the biological community to improve topological models.

Available diet studies generally present important biases in resolution that affect the realism of food webs. It would be very important to clarify the diet of many species, especially for prey taxa that are usually aggregated into large groups, such as macroalgae, phytoplankton and zooplankton. Food web studies based on stable isotopes will add important information to the current knowledge on feeding interaction in the marine realm. Feeding behaviour studies will certainly, also, give important contributions to this field.

Topological studies, like the ones presented in these work, are often the only option given data scarcity and are a best-case scenario that only accounts for the minimum number of secondary extinctions, so efforts should be put in place to allow dynamic approaches in the study of the robustness of food webs. This implies an important investment in collecting data on body size, body mass, abundance and interaction strength. Such data would allow the repetition of the removal exercises conducted in the present work within a dynamic approach.

Furthermore, other removal criteria based on realistic vulnerability rankings of species towards a stressor (e.g. acidification, hypoxia, oil contamination) should be used so that their impact on food webs can be simulated in topological and dynamic approaches. For this to be possible, the species' vulnerability to stressors must be tested so that vulnerability rankings are available for food web research. This will require a major joint effort from field and experimental biologists, as well as from food web modelers.

From an evolutionary perspective, several questions regarding the ability of organisms to adapt and thrive in a changing ocean remain to be answered. The ability for species to change their diet and their potential for adaptation to warming, is one of these issues. Acclimation and genetic adaptation as a means of coping with rising environmental temperatures shall play a key role in the persistence of communities in the face of global warming. Studies that investigate the role of trans-generational plasticity, parental effects (e.g. mitochondrial DNA inherited from the female) and other epigenetic phenomena (DNA methylation) in the maintenance and viability of populations will surely help to untangle species' potential for adaptation to warming.

This work also showed that no seasonal variation was detected in the food web network structure of intertidal rock pools. However, the main characteristic of the analysis of food web networks' structure is that it is conducted using only data on presence/absence of taxa and occurrence of feeding links. So, although seasonality in the food web topology was not detected in present thesis, it does not mean that these food webs do not suffer important seasonal changes in terms of species biomass and energy transfer. The present work exposes the basic topology of these webs and is, thus, a first step in this investigation, but future studies using allometric food web models are needed and will certainly bring new insights into the seasonality of the food webs of intertidal rock pools in temperate regions.

It was concluded that intertidal rock pools are likely important feeding grounds for transient juvenile marine fish. Since, these are early stages of important commercial species, a special attention should be given to them. Their trophic dependence on pools should be evaluated, so that the importance of this habitat in these species' lifecycle is fully understood. Intertidal rock pools attain very high temperatures in the tropics, acting as ecological traps for some species, this way the significance of such events for the species' populations and fishing stocks should be investigated.

Lastly, the usefulness of intertidal rock pools has been demonstrated in this thesis, but the entire intertidal zone is important and will probably be one of the habitats where climate change will strike first, future studies should therefore seize the opportunity to detect and manage the effects of climate change, using rocky shores as sentinel ecosystems.

ANNEXES

ANNEX 1

PhD outputs

Articles in international peer-reviewed journals

Mendonça, V., Madeira, C., Dias, M., Vermandele, F., Archambault, P., Dissanayake, A., ... & Vinagre, C. (2018). What's in a tide pool? Just as much food web network complexity as in large open ecosystems. *PloS one*, 13(7), e0200066. DOI: 10.1371/journal.pone.0200066

Mendonça, V., Madeira, C., Dias, M., Silva, A.C.F., Flores, A.A.V, Vinagre, C. Robustness of temperate and tropical tide pool food webs: comparing species trait-based sequential deletions (submitted).

Mendonça, V., Madeira, C., Dias, M., Silva, A.C.F., Flores, A.A.F., Vinagre, C. Robustness of food web complex networks to heatwaves in tropical and temperate shallow waters (submitted).

Mendonça, V., Cereja, R. Dias, M., Flores, A.A.V., Vinagre, C. Seasonal Variation in the Food Web Network Structure of Intertidal Rock Pools. (submitted)

Mendonça, V., Flores, A. A., Silva, A. C., & Vinagre, C. (2019). *Estuarine, Coastal and Shelf Science*, 106255. DOI: 10.1016/j.ecss.2019.106255.

Posters presented in scientific meetings (national and international)

2019. Mendonça V, Madeira C, Dias M, Silva A, Flores AAV, Vinagre C. Robustness of tropical and temperate food web networks to species removals. ICYMARE – International Conference for Young Marine Researchers, 24th-27th September, Bremen, Germany.

Oral communications in scientific meetings (national and international)

Vinagre, C., Mendonça, V., Madeira, C. Dias, M. Silva, A.C.F., Flores, A.A.V. (2018). What happens when thermally vulnerable species are removed from food web networks?. *International Meeting on Marine Research 2018.*

ANNEX 2

Supplementary material for chapter 2

Table SM2.1. General characteristics of the pools surveyed.

	Nº pools	TºC (mean)	Salinity (mean)	Area (m^2) range	Depth (m) (mean)	Distance to sea (m) (mean)	Height (m) (mean)
Canada	28	16.9	26.3	0.19-3.90	0.15	11.4	1.4
UK	8	21.1	33.0	0.17-5.40	0.22	5.0	1.0
Portugal-west coast	32	19.6	35.1	0.16-14.70	0.30	9.7	1.1
Portugal-Madeira	14	21.6	36.6	0.40-13.79	0.34	3.9	1.3
Brazil - SP	18	29.4	33.9	0.30-32.50	0.35	2.1	0.7
Brazil - CE	16	32.0	37.0	0.16-18.78	0.32	14.7	0.3

Table SM2.2. List of all taxa identified in the pools.

	Taxa
Canada (Gulf St. Lawrence)	Acari
	Alaria esculenta
	Alitta virens
	Antithamnion sp.
	Ascophyllum nodosum
	Aulactinia stella
	Balanus crenatus
	Cancer irroratus
	Chordaria flagelliformis
	Clathromorphum circumscriptum
	Coilodesme bulligera
	Complexe fucus
	Devaleraea ramentacea
	Diatomophycea
	Dictyosiphon foeniculaceus
	Eteone longa
	Eteone sp.
	Fabricia stellaris
	Gammarus oceanicus
	Gayralia oxysperma
	Harpacticoida
	Hediste diversicolor
	Hildenbrandia rubra
	Hildenbrandia rubra
	Insecta
	Jaera (Jaera) albifrons
	Lithophyllum sp.
	Littorina littorea
	Littorina obtusata
	Littorina saxatilis
	Macoma balthica
	Monostroma grevillei
	Mytilus sp.
	Naineris quadricuspida
	Nematoda
	Oligochaeta
	Petalonia fascia
	Pholoe minuta
	Pholoe sp.

	Phytoplankton
	Polychaeta
	Polydora sp.
	Polydora websteri
	Polysiphonia sp.
	Ralfsia fungiformis
	Ralfsia verrucosa
	Rhodomela confervoides
	Rhodomela lycopodioides
	Saccharina latissima
	Scytosiphon lomentaria
	Sertulariidae
	Skeneopsis planorbis
	Strongylocentrotus droebachiensis
	Testudinalia testudinalis
	Ulvaria obscura
	Wildemania miniata
	Zooplankton
UK	*Acanthochitona crinita*
	Actinia equina
	Amphipholis squamata
	Ampithoidae
	Anemonia viridis
	Ascophyllum nodosum
	Asterina gibbosa
	Austrominius modestus
	Barleeia sp.
	Bivalvia
	Cancer pagurus
	Carcinus maenas
	Chondrus crispus
	Chorda filum
	Colpomenia peregrina
	Corallina officinalis
	Cumacea
	Desmarestia aculeata
	Dictyota dichotoma
	Ectocarpus siliculosus
	Fucus serratus
	Fucus vesiculosus
	Furcellaria lumbricalis

Gammaridae
Gibbula cineraria
Gibbula umbilicalis
Gobius paganellus
Halacaridae
Halurus equisetifolius
Harparticoida
Insecta
Jania rubens
Lineus ruber
Lithophyllum incrustans
Littorina Littorea
Littorina saxatilis
Lomentaria articulata
Mastocarpus stellatus
Membranipora membranaceae
Mysida
Mytilus galloprovincialis
Nassariidae
Nemalion helminthoides
Nematoda
Nemertea
Nereididae
Nucella lapillus
Nymphon gracile
Oligochaeta
Omalogyra atomus
Ophiocomina nigra
Oshurkovia littoralis
Ostracoda
Pagurus bernhardus
Palaemon elegans
Palaemon serratus
Palmaria palmata
Patella depressa
Patella ulyssiponensis
Patella vulgata
Phorcus lineatus
Phymatolithon calcareum
Phytoplankton
Polychaeta

	Polysiphonia sp.
	Pomatoschistus minutus
	Procerodes littoralis
	Rhodomela confervoides
	Rissoidae
	Saccharina latissima
	Sargassum muticum
	Solenocurtus strigilatus
	Sphaeromatidae
	Spirorbis spirorbis
	Stenothoidae
	Symphodus melops
	Tanaidacea
	Taurulus bubalis
	Tellinidae
	Tonicella rubra
	Turritellidae
	Ulva intestinalis
	Ulva lactuca
	Ulva linza
	Venerupis sp.
	Zooplankton
Portugal (west coast)	*Acanthochitona crinita*
	Achelia echinata
	Actinia
	Actinia equina
	Actinia fragacea
	Actinothoe sphyrodeta
	Aeolidia papillosa
	Ammotheidae
	Amphilochidae
	Amphipholis squamata
	Amphipoda
	Ampithoe valida
	Ampithoidae
	Anemonia sulcata
	Angulus tenuis
	Anthura sp.
	Aplysia punctata
	Asparagopsis armata
	Asterina gibbosa

	Atherina boyeri
	Aulactinia verrucosa
	Balanus sp.
	Barleeia sp.
	Barnea candida
	Bifurcaria bifurcata
	Botryllus schlosseri
	Callianassidae
	Calliopidae
	Calliostoma zizyphinum
	Caprella linearis
	Caprella sp.
	Carcinus maenas
	Cardiidae
	Cardium papillosum
	Ceramium ciliatum
	Ceramium virgatum
	Chaetogammarus sp.
	Chiton (Rhyssoplax) olivaceus
	Chondrus crispus
	Chrysallida pellucida
	Chthamalus sp.
	Cladophora rupestris
	Codium sp.
	Coleoptera
	Colpomenia peregrina
	Corallina officinalis
	Coryphoblennius galerita
	Cumacea
	Cymodoce truncata
	Cystoseira
	Diaphorodoris papillata
	Dictyopteris polypodioides
	Dictyota dichotoma
	Diplodus sargus sargus
	Egg *Sepia officinalis*
	Ellisolandia elongata
	Endeis spinosa
	Epitonium clathrus
	Eriphia verrucosa
	Eulalia viridis

Felimida purpurea
Fucus vesiculosus
Gammaridae
Gammarus sp.
Gelidium corneum
Gelidium spinosum
Gibbula umbilicalis
Gobius niger
Gobius paganellus
Gracilariopsis longissima
Halacaridae
Harparticoida
Heterosiphonia sp.
Hippolyte varians
Holothuria sp.
Hydrobia sp.
Hypselodoris sp.
Idoteidae
Insecta
Iphimediidae
Ischyroceridae
Jaera sp.
Jania sp.
Laurencia pinnatifida
Leathesia marina
Lepadogaster lepadogaster
Lepidochitona cinerea
Lepidotrigla
Leptochiton algesirensis
Leucothoidae
Lipophrys pholis
Lipophrys trigloides
Lithophyllum
Lithophyllum byssoides
Littorina sp.
Liza ramada
Lophozozymus incisus
Lysianassidae
Maja squinado
Marthasterias glacialis
Melitidae

	Modiolula phaseolina
	Munnidae
	Muricidae
	Musculus costulatus
	Mysida
	Mytilus galloprovincialis
	Nassariidae
	Nassarius reticulatus
	Necora puber
	Nematoda
	Nemertea
	Nereididae
	Nucella sp.
	Nymphonidae
	Ocenebra erinaceus
	Octopus vulgaris
	Oligochaeta
	Omalogyra atomus
	Onchidella celtica
	Ophiuroidae
	Pachygrapsus marmoratus
	Padina pavonica
	Palaemon adspersus
	Palaemon elegans
	Palaemon longirostris
	Palaemon serratus
	Palmaria palmata
	Pantopoda
	Paracentrotus lividus
	Patella depressa
	Patella rustica
	Patella ulyssiponensis
	Patella vulgata
	Petricolinae
	Phorcus lineatus
	Phytoplankton
	Pirimela denticulata
	Polinices sp.
	Polychaeta
	Porcellana platycheles
	Porphyra sp.

	Procedores sp.
	Pyramidellidae
	Rhodymenia pseudopalmata
	Rissoidae
	Sabellaria alveolata
	Saccorhiza polyschides
	Sagartia elegans
	Sardina pilchardus
	Sargassum sp.
	Setia ugesae
	Skeneidae
	Solenocurtus strigilatus
	Sphacelaria cirrosa
	Sphaeromatidae
	Spirobranchus sp.
	Spirorbis spirorbis
	Stenothoidae
	Tanaidacea
	Tricolia pullus
	Turbellaria
	Turbonilla lactea
	Turridae
	Turritella sp.
	Turritellidae
	Turtonia minuta
	Ulva lactuca
	Venerupis sp.
	Xantho pilipes
	Zooplankton
Portugal: Madeira	*Acanthochitona crinita*
	Acanthonyx lunulatus
	Ammotheidae
	Amphipholis squamata
	Ampithoidae
	Anemonia sargassensis
	Barleeia sp.
	Batzella inops
	Caprella sp.
	Cardiidae
	Caulerpa webbiana
	Ceramium sp.

	Cladophora prolifera
	Cladostephus spongiosus
	Codium adhaerens
	Codium sp.
	Corallina sp.
	Coralliophila meyendorffii
	Coryphoblennius galerita
	Cymodoce truncata
	Cystoseira abies-marina
	Dictyopteris polypodioides
	Dictyota bartayresiana
	Dictyota sp.
	Eriphia verrucosa
	Eulalia viridis
	Gammaridae
	Gibbula pennanti
	Gibbula umbilicalis
	Gobius sp.
	Grapsus adscensionis
	Halopteris filicina
	Harparticoida
	Hypnea sp.
	Insecta
	Isopoda
	Laurencia sp.
	Lepadogaster zebrina
	Leucothoidae
	Liagora sp.
	Lipophrys pholis
	Lithophyllum
	Liza ramada
	Lophozozymus incisus
	Lysianassidae
	Nematoda
	Oligochaeta
	Omalogyra atomus
	Ostracoda
	Pachygrapsus transversus
	Padina pavonica
	Palaemon elegans
	Palaemon sp.

		Parablennius parvicornis
		Paracentrotus lividus
		Patella ulyssiponensis
		Petaloconchus sp.
		Phorcus lineatus
		Phytoplankton
		Pirimela denticulata
		Polychaeta
		Priapulida
		Rhodomelaceae
		Sargassum vulgare
		Serpulidae
		Spirobranchus sp.
		Spirorbis spirorbis
		Stenothoidae
		Stramonita haemastoma
		Stypocaulon scoparium
		Tanaidacea
		Thalassoma pavo
		Tricleocarpa cylindrica
		Tricolia sp.
		Turridae
		Ulva sp.
		Valonia sp.
		Zooplankton
	Brazil-SP	*Acanthophora spicifera*
		Alpheus formosus
		Amphiroa sp.
		Asteronema breviarticulatum
		Bachelotia sp.
		Barbatia candida
		Bathygobius soporator
		Bostrychia sp.
		Bryopsis sp.
		Bunodosoma caissarum
		Bunodosoma cangicum
		Callinectes sapidus
		Carcinus sp.
		Caulerpa racemosa
		Cerithium atratum

	Chaetomorpha gracilis
	Clibanarius vittatus
	Codium sp.
	Colomastigidae
	Colpomenia peregrina
	Columbellidae
	Copepoda
	Crassostrea virginica
	Cronius ruber
	Cumacea
	Cuspidariidae
	Dictyota dichotoma
	Dictyota sp.
	Echinometra lucunter
	Eriphia gonagra
	Eucinostomus melanopterus
	Eurypanopeus abreviatus
	Fissurella clenchi
	Gammaridae
	Gelidium sp.
	Gnatostnaetroidae
	Harpaticoida
	Holothuria (Halodeima) grisea
	Hymeniacidon heliophila
	Hypnea musciformis
	Idotea
	Ischnochiton striolatus
	Isognomon bicolor
	Isopoda
	Jania rubens
	Lischkeia
	Lithophyllum
	Litopenaeus schmitti
	Lottia subrugosa
	Malacoctenus delalandii
	Megabalanus tintinnabulum
	Metidae
	Microphrys bicornutus
	Morula nodulosa
	Mytilaster solisianus

	Nematoda
	Nodilittorina lineolata
	Odontesthes argentinensis
	Oedicerotidae
	Ophiactis savignyi
	Pachygrapsus transversus
	Padina sp.
	Pagurus sp.
	Palaemon northropi
	Parablennius marmoreus
	Perna perna
	Petaloconchus sp.
	Phragmatopoma caudata
	Phytoplankton
	Platlyhelminthes
	Polychaeta
	Potamididae
	Protopalythoa variabilis
	Renilla sp
	Rhizoclonium riparium
	Sargassum cymosum
	Scartella cristata
	Sebidae
	Serpulidae
	Solenocurtus strigilatus
	Sphacelaria sp
	Spirobranchus sp.
	Stramonita haemastoma
	Strombus sp.
	Tanaidacea
	Tegula viridula
	Tetraclita stalactifera
	Ulva lactuca
	Zooplankton
Brazil-CE	*Abudefduf saxatilis*
	Acanthophora spicifera
	Amphiroa sp.
	Ampithoidae
	Anemonia sargassensis
	Aplysia dactylomela

	Astyris lunata
	Aurantilaria aurantiaca
	Bathygobius soporator
	Bittiolum varium
	Boonea jadisi
	Bostrychia sp.
	Brachidontes sp.
	Bryopsis pennata
	Bryopsis plumosa
	Bryopsis sp.
	Callinectes ornatus
	Callinectes sp.
	Calliopidae
	Caulerpa cupressoides
	Caulerpa prolifera
	Caulerpa racemosa
	Caulerpa scalpelliformis
	Caulerpa sp.
	Centroceras clavulatum
	Cerithium atratum
	Chaetomorpha gracilaris
	Chondria curvilineata
	Chthamalus bisinuatus
	Cinachyrella alloclada
	Clibanarius antillensis
	Codium decorticatum
	Columbella mercatoria
	Corallina sp.
	Cryptonemia crenulata
	Cumacea
	Cystodytes dellechiajei
	Dictyopteris delicatula
	Dictyota menstrualis
	Dictyota mertensii
	Dictyota sp.
	Didemnum granulatum
	Diplodonta sp.
	Engina turbinella
	Epialtus brasiliensis
	Epitonium sp.
	Eriphia gonagra

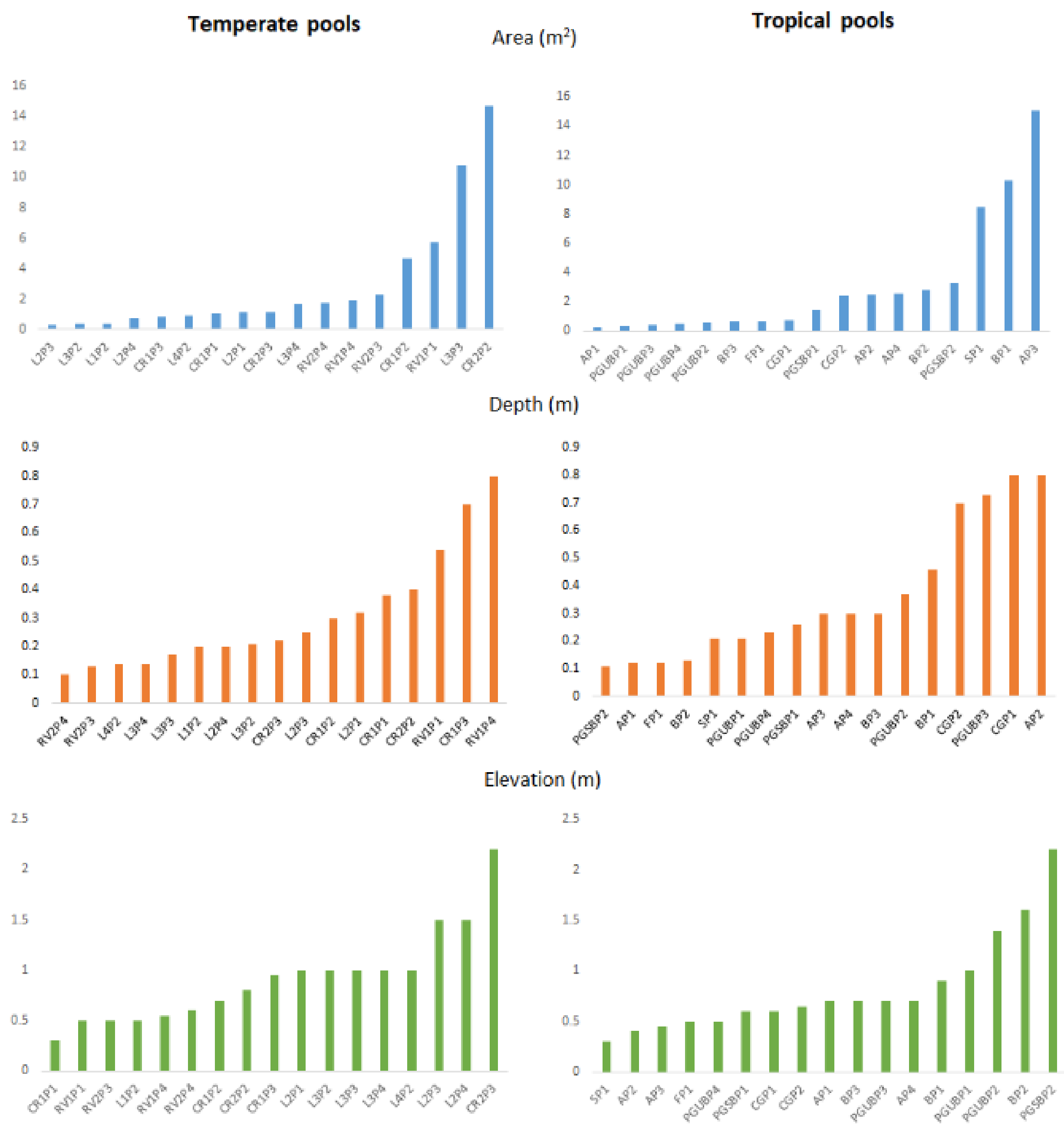
	Eudistoma vannamei
	Eulithidium affine
	Fissurella rosea
	Gammaridae
	Gelidium pusillum
	Gracilaria Birdiae
	Gracilaria domingensis
	Gracilaria sp.
	Haemulon parra
	Haliclona sp.
	Harpaticoida
	Hippolyte obliquimanus
	Hypnea musciformis
	Idotea
	Idoteidae
	Isaurus sp.
	Ischnochiton striolatus
	Ischnoplax pectinata
	Isopoda
	Leptopecten sp.
	Lithophyllum sp.
	Lithopoma phoebium
	Lobophora variegata
	Lysmata sp.
	Mangelia sp.
	Menippe nodifrons
	Mycale arcuiris
	Mycale sp.
	Nematoda
	Neogonodactylus sp.
	Odostomia sp.
	Oligochaeta
	Olivancillaria sp.
	Ophiura sp.
	Ophiuroidea
	Ostracoda
	Pachygrapsus transversus
	Padina gymnospora
	Pagurus sp.
	Palaemon northropi
	Panopeus herbstii

	Panopeus sp.
	Phallusia nigra
	Phytoplankton
	Plocamium brasiliense
	Plocamium sp.
	Polychaeta
	Polysyncraton amethysteum
	Pomacanthus paru
	Protopalythoa variabilis
	Sabellidae
	Sargassum cymosum
	Sargassum vulgare
	Scartella cristata
	Siderastea sp.
	Sipunculidae
	Spatoglossum schroederi
	Sphacelaria sp.
	Sphoeroides sp.
	Spirorbis sp.
	Stenothoidae
	Stramonita haemastoma
	Talitridae
	Tanaidacea
	Tedania sp.
	Tegula viridula
	Turbellaria
	Ulva lactuca
	Valonia aegagropila
	Zoanthus sociatus
	Zooplankton

ANNEX 3

Supplementary material for chapter 3

Figure SM3.1. Intertidal rock pools selected in temperate and tropical region with similar surface area, depth and elevation.



ANNEX 4

Supplementary material for chapter 5

Table SM5.1. The main pool characteristics in each season.

Season	Beach	Average depth (m)	Temp. (ºC)	Salinity (‰)
Autumn	Cabo Raso	13.71	17.68	36.50
	Raio Verde	29.88	17.59	36.75
	Paimogo	25.75	18.51	37.38
	Peralta	27.25	18.08	36.50
Winter	Cabo Raso	22.38	15.24	35.13
	Paimogo	30.88	15.38	34.31
	Peralta	29.13	13.16	35.25
	Raio Verde	28.75	14.20	35.13
Spring	Cabo Raso	24.50	14.50	34.00
	Paimogo	30.50	13.40	35.00
	Peralta	31.63	14.99	32.19
	Raio Verde	29.00	14.46	32.48
Summer	Cabo Raso	24.00	15.01	32.06
	Paimogo	31.14	16.11	31.94
	Peralta	30.88	14.88	34.11
	Raio Verde	28.50	14.63	33.25

Table SM5.2. List of all taxa identified in the pools for each season.

Autumn (N = 141)	**Winter** (N = 122)	**Spring** (N = 127)	**Summer** (N = 140)
Ulva lactuca	*Ulva lactuca*	*Ulva lactuca*	*Ulva lactuca*
Codium sp.	*Codium* sp.	*Codium* sp.	*Codium* sp.
Colpomenia peregrina	*Colpomenia peregrina*	*Cladophora rupestris*	*Cladophora rupestris*
Bifurcaria bifurcata	*Bifurcaria bifurcata*	*Colpomenia peregrina*	*Enteromorpha sp.*
Cystoseira sp.	*Cystoseira sp.*	*Bifurcaria bifurcata*	*Chaetomorpha sp.*
Sargassum sp.	*Sargassum* sp.	*Cystoseira sp.*	*Colpomenia peregrina*
Fucus vesiculosus	*Fucus vesiculosus*	*Sargassum* sp.	*Bifurcaria bifurcata*
Sphacelaria cirrosa	*Sphacelaria cirrosa*	*Fucus vesiculosus*	*Cystoseira sp.*
Saccorhiza polyschides	*Saccorhiza polyschides*	*Sphacelaria cirrosa*	*Sargassum* sp.
Dictyota dichotoma	*Dictyota dichotoma*	*Saccorhiza polyschides*	*Sphacelaria cirrosa*
Dictyopteris polypodioides	*Asparagopsis armata*	*Dictyota dichotoma*	*Dictyota dichotoma*
Padina pavonica	*Lithophyllum sp.*	*Asparagopsis armata*	*Leathesia marina*
Asparagopsis armata	*Mesophyllum sp.*	*Ellisolandia elongata*	*Dictyopteris polypodioides*
Plocamium cartilagineum	*Corallina officinalis*	*Lithophyllum sp.*	*Asparagopsis armata*
Lithophyllum sp.	*Ceramium ciliatum*	*Mesophyllum sp.*	*Lithophyllum sp.*
Mesophyllum sp.	*Aglaothamnion sp.*	*Corallina officinalis*	*Mesophyllum sp.*
Jania sp.	*Laurencia pinnatifida*	*Ceramium ciliatum*	*Corallina officinalis*
Corallina officinalis	*Palmaria palmata*	*Laurencia pinnatifida*	*Ceramium ciliatum*
Ceramium ciliatum	*Chondrus crispus*	*Palmaria palmata*	*Laurencia pinnatifida*
Hypnea sp.	*Gelidium corneum*	*Chondrus crispus*	*Palmaria palmata*
Laurencia pinnatifida	*Gelidium spinosum*	*Gelidium corneum*	*Gelidium corneum*
Palmaria palmata	*Mastocrapo stellatos*	*Gelidium spinosum*	*Gelidium spinosum*
Chondrus crispus	Porifera	*Mastocrapo stellatos*	*Ceramium virgatum*
Gelidium corneum	*Aulactinia verrucosa*	Porifera	*Polysiphonia sp.*
Gelidium spinosum	*Anemonia sulcata*	*Aulactinia verrucosa*	*Pterosiphonia complanata*
Mastocrapo stellatos	*Actinia equina*	*Anemonia sulcata*	*Boergeseniella sp.*
Porifera	*Actinia fragacea*	*Actinia equina*	*Aglaothamnion sp.*
Aulactinia verrucosa	*Actinothoe sphyrodeta*	*Actinia fragacea*	*Caulacanthus sp.*
Anemonia sulcata	*Sagartia elegans*	*Actinothoe sphyrodeta*	*Gracilaria sp.*
Actinia equina	Turbellaria	*Sagartia elegans*	*Plocamium sp.*
Actinia fragacea	Nemertea	Turbellaria	*Mastocrapo stellatos*
Actinothoe sphyrodeta	Nematoda	Nemertea	Porifera
Sagartia elegans	Capitellidae	*Lineus longissimus*	*Aulactinia verrucosa*
Nematoda	Serpulidae	Nematoda	*Anemonia sulcata*
Capitellidae	Nereididae	Capitellidae	*Actinia equina*
Opheliidae	*Eulalia viridis*	Serpulidae	*Actinia fragacea*
Nereididae	Cirratulidae	Nereididae	*Actinothoe sphyrodeta*
Eulalia viridis	*Spirorbis spirorbis*	*Eulalia viridis*	*Sagartia elegans*
Spirobranchus sp.	*Pamatoceros sp.*	Cirratulidae	Turbellaria

Spirorbis spirorbis	*Sabellaria alveolata*	Orbiniidae	Nemertea
Pamatoceros sp.	*Acanthochitona crinita*	Syllidae	Nematoda
Sabellaria alveolata	*Lepidochitona cinerea*	Glyceridae	Capitellidae
Terebellidae	*Leptochiton algesirensis*	Nephtyidae	Serpulidae
Acanthochitona crinita	*Patella vulgata*	Pholoidae	Amphinomidae
Lepidochitona cinerea	*Patella depressa*	*Spirobranchus sp.*	Nereididae
Chiton olivaceus	*Patella ulyssiponensis*	*Spirorbis spirorbis*	*Eulalia viridis*
Leptochiton algesirensis	*Fissurella sp.*	*Pamatoceros sp.*	Cirratulidae
Patella vulgata	*Gibbula umbilicalis*	*Sabellaria alveolata*	Orbiniidae
Patella depressa	*Phorcus lineatus*	*Acanthochitona crinita*	Pholoidae
Patella ulyssiponensis	*Manzonia sp.*	*Lepidochitona cinerea*	*Spirorbis spirorbis*
Alvania sp.	*Pusillina inconspicua*	*Leptochiton algesirensis*	*Pamatoceros sp.*
Skeneidae	*Rissoa parva*	*Patella vulgata*	*Sabellaria alveolata*
Gibbula umbilicalis	*Ocenebra erinaceus*	*Patella depressa*	*Acanthochitona crinita*
Phorcus lineatus	*Nassarius reticulatus*	*Patella ulyssiponensis*	*Lepidochitona cinerea*
Manzonia sp.	*Tricolia pullus*	*Gibbula umbilicalis*	*Leptochiton algesirensis*
Setia ugesae	*Omalogyra atomus*	*Phorcus lineatus*	*Patella vulgata*
Pusillina inconspicua	*Pyramidellidae*	*Setia ugesae*	*Patella depressa*
Rissoa parva	*Barleeia* sp.	*Pusillina inconspicua*	*Patella ulyssiponensis*
Nucella sp.	*Littorina* sp.	*Rissoa parva*	*Gibbula umbilicalis*
Ocenebra erinaceus	*Melarhaphe neritoides*	*Ocenebra erinaceus*	*Phorcus lineatus*
Nassariidae	*Epitonium clathrus*	*Nassarius reticulatus*	*Setia ugesae*
Nassarius reticulatus	*Aplysia punctata*	*Tricolia pullus*	*Pusillina inconspicua*
Tricolia pullus	*Cerithiopsis sp.*	*Omalogyra atomus*	*Rissoa parva*
Ondina sp.	*Eatonina sp.*	*Odostomia eulynoides*	*Ocenebra erinaceus*
Omalogyra atomus	*Felimida purpurea*	*Barleeia* sp.	*Nassarius reticulatus*
Turbonilla lactea	*Musculus costulatus*	*Littorina* sp.	*Tricolia pullus*
Chrysallida pellucida	*Mytilus galloprovincialis*	*Stramonita haemas-toma*	*Omalogyra atomus*
Barleeia sp.	*Hiatella sp.*	*Turbonilla lactea*	*Odostomia eulynoides*
Littorina sp.	*Cardita sp.*	*Aplysia punctata*	*Barleeia* sp.
Melarhaphe neritoides	Cardiidae	*Cerithiopsis sp.*	*Littorina sp.*
Epitonium clathrus	*Parvicardium sp.*	*Eatonina sp.*	*Manzonia sp.*
Aplysia punctata	*Solecurtus strigilatus*	*Aeolidia papillosa*	*Liomesus sp.*
Odostomia eulynoides	*Venerupis* sp.	*Musculus costulatus*	*Philbertia sp.*
Naticidae	*Lasaea sp.*	*Mytilus galloprovincialis*	*Calliostoma zizyphinum*
Cerithiopsis sp.	*Octopus vulgaris*	*Hiatella sp.*	*Chrysallida pellucida*
Eatonina sp.	Cumacea	Cardiidae	*Ondina sp.*
Fossarus sp.	Tanaidacea	*Parvicardium sp.*	Naticidae
Aeolidia papillosa	Mysida	*Solecurtus strigilatus*	*Epitonium clathrus*
Hypselodoris sp.	*Dynamene sp.*	*Venerupis* sp.	*Aplysia punctata*
Musculus costulatus	*Cymodoce truncata*	*Lasaea sp.*	*Cerithiopsis sp.*
Mytilus galloprovincialis	Podoceridae	*Octopus vulgaris*	*Eatonina sp.*

Petricolinae	Idoteidae	Tanaidacea	*Diaphorodoris papillata*
Cardiidae	Sebidae	Kalliapseudidae	*Musculus costulatus*
Parvicardium sp.	Ampithoidae	*Dynamene sp.*	*Mytilus galloprovincialis*
Solecurtus strigilatus	Gammaridae	*Cymodoce truncata*	Cardiidae
Venerupis sp.	Stenothoidae	Idoteidae	*Cardita calyculata*
Lasaea sp.	Calliopidae	Jaera sp.	*Parvicardium sp.*
Octopus vulgaris	Colomastigidae	Cirolanidae	*Solecurtus strigilatus*
Tanaidacea	Corophiidae	*Anthura sp.*	*Venerupis* sp.
Mysida	Hyalidae	Sebidae	*Lasaea sp.*
Dynamene sp.	*Caprella* sp.	Ampithoidae	*Octopus vulgaris*
Cymodoce truncata	Melitidae	Gammaridae	Tanaidacea
Aoridae	*Palaemon serratus*	Stenothoidae	*Dynamene sp.*
Idoteidae	*Palaemon elegans*	Calliopidae	*Cymodoce truncata*
Anthura sp.	*Lophozozymus incisus*	Colomastigidae	Idoteidae
Sebidae	*Pirimela denticulata*	Corophiidae	*Anthura sp.*
Ampithoidae	*Carcinus maenas*	*Caprella* sp.	Sebidae
Gammaridae	*Porcellana platycheles*	Melitidae	Ampithoidae
Stenothoidae	*Necora puber*	*Palaemon elegans*	Gammaridae
Calliopidae	*Anapagurus laevis*	*Lophozozymus incisus*	Stenothoidae
Colomastigidae	*Pagurus bernhardus*	*Pirimela denticulata*	Corophiidae
Corophiidae	*Cancer pagurus*	*Carcinus maenas*	*Caprella* sp.
Hyalidae	Ostracoda	*Porcellana platycheles*	Melitidae
Caprella sp.	Harparticoida	*Necora puber*	Leptocheliidae
Melitidae	Peltidiidae	*Anapagurus laevis*	Lysianassidae
Palaemon serratus	Metidae	*Pollicipes pollicipes*	*Palaemon serratus*
Palaemon elegans	*Chthamalus* sp.	Ostracoda	*Palaemon elegans*
Hippolyte varians	Insecta	Harparticoida	*Eriphia verrucosa*
Eriphia verrucosa	Halacaridae	Peltidiidae	*Pachygrapsus marmoratus*
Pachygrapsus marmora-tus	Pycnogonida	Metidae	*Xantho pilipes*
Lophozozymus incisus	*Asterina gibbosa*	*Chthamalus* sp.	*Maja squinado*
Xantho pilipes	*Marthasterias glacialis*	*Balanus sp.*	*Lophozozymus incisus*
Pirimela denticulata	Ophiuroidea	Insecta	*Pirimela denticulata*
Carcinus maenas	*Amphipholis squamata*	Tipulidae	*Carcinus maenas*
Porcellana platycheles	*Paracentrotus lividus*	Halacaridae	*Porcellana platycheles*
Maja squinado	*Botryllus schlosseri*	Pycnogonida	*Necora puber*
Necora puber	*Syngnathus acus*	*Asterina gibbosa*	*Anapagurus laevis*
Anapagurus laevis	*Lipophrys trigloides*	*Marthasterias glacialis*	Ostracoda
Ostracoda	*Lipophrys pholis*	Ophiuroidea	Harparticoida
Harparticoida	*Coryphoblennius gale-rita*	*Paracentrotus lividus*	Peltidiidae
Metidae	*Gobius paganellus*	*Holothuria sp.*	Metidae

Chthamalus sp.	*Lepadogaster lepadogaster*	*Botryllus schlosseri*	*Chthamalus* sp.
Insecta		*Conger conger*	*Balanus sp.*
Halacaridae		*Lipophrys pholis*	Insecta
Pycnogonida		*Coryphoblennius galerita*	Halacaridae
Asterina gibbosa		*Gobius paganellus*	Pycnogonida
Marthasterias glacialis		*Lepadogaster lepadogaster*	*Asterina gibbosa*
Ophiuroidea			*Marthasterias glacialis*
Paracentrotus lividus			Ophiuroidea
Botryllus schlosseri			*Amphipholis squamata*
Conger conger			*Paracentrotus lividus*
Liza ramada			*Holothuria sp.*
Syngnathus acus			*Botryllus schlosseri*
Diplodus cervinus			*Conger conger*
Lipophrys trigloides			*Diplodus sargus sargus*
Lipophrys pholis			*Symphodus sp.*
Coryphoblennius galerita			*Lipophrys pholis*
Gobius niger			*Coryphoblennius galerita*
Gobius paganellus			*Gobius paganellus*
Symphodus melops			*Lepadogaster lepadogaster*
Lepadogaster lepadogaster			

Table SM5.3. Compilation of statistical analyses (factorial ANOVAs) on the food web network properties analysed and seasons. Significant results are presented in red.

	Seasons				
	SS	Degr. Of Freedom	MS	F	P
S	632.4	3	210.8	5.275	.002*
L/S	9.539	3	3.18	5.117	.002*
C	0.01	3	0.003	4.763	.004*
T	0.011	3	0.004	1.03	0.382
I	0.017	3	0.006	0.949	0.419
B	0.013	3	0.004	2.127	0.1
H	0.005	3	0.002	0.725	0.539
GenSD	0.09	3	0.03	2.716	.048*
VulSD	0.204	3	0.068	4.598	.004*
LinkSD	0.087	3	0.029	5.451	.001*
TL	0.039	3	0.013	1.914	0.131
Chain	0.013	3	0.004	2.127	0.1
Omn	0.003	3	0.001	0.384	0.765
Can	0.005	3	0.002	0.893	0.447
Path	0.065	3	0.022	4.52	.005*
Clust	0.005	3	0.002	2.631	0.053
Resource count	551.7	3	183.9	4.626	.004*
Consumer Count	535.1	3	178.4	5.029	.003*

Table SM5.4. Summary results of factorial ANOVAs showing main effects of seasons (Autumn, Winter, Spring and Summer) on food web network properties analysed. Significant results (p-value < 0.05) are presented in red.

	Autumn					Winter					Spring					Summer				
	SS	Degr. Of Freedom	MS	F	P	SS	Degr. Of Freedom	MS	F	P	SS	Degr. Of Freedom	MS	F	P	SS	Degr. Of Freedom	MS	F	P
S	609.3	3	203.1	5	.007*	264.3	3	88.11	1.984	0.139	460.6	3	153.5	12.98	.000*	212.3	3	70.78	2.844	0.056
L/S	1.032	3	0.344	0.4	0.754	0.807	3	0.269	0.31	0.818	6.982	3	2.327	7.827	.001*	0.406	3	0.135	0.342	0.795
C	0.011	3	0.004	4.991	.007*	0.009	3	0.003	6.823	.001*	0.006	3	0.002	3.861	.020*	0.005	3	0.002	5.14	.006*
T	0.005	3	0.002	0.506	0.682	0.002	3	0.001	0.137	0.937	0.006	3	0.002	0.472	0.704	0.008	3	0.003	1.754	0.179
I	0.005	3	0.002	0.215	0.885	0.01	3	0.003	0.349	0.79	0.033	3	0.011	1.743	0.181	0.003	3	0.001	0.449	0.72
B	0.003	3	0.001	0.185	0.905	0.005	3	0.002	1.107	0.363	0.027	3	0.009	10.8	.000*	0.006	3	0.002	3.715	.023*
H	0.003	3	0.001	0.456	0.715	0.003	3	0.001	0.404	0.751	0.018	3	0.006	3.563	.027*	0.009	3	0.003	1.489	0.239
GenSD	0.171	3	0.057	2.154	0.116	0.03	3	0.01	1.339	0.282	0.017	3	0.006	1.734	0.183	0.009	3	0.003	0.831	0.488
VulSD	0.096	3	0.032	2.009	0.135	0.14	3	0.047	5.666	.004*	0.017	3	0.006	1.734	0.183	0.192	3	0.064	7.342	.001*
LinkSD	0.11	3	0.037	5.264	.005*	0.065	3	0.022	7.795	.001*	0.048	3	0.016	6.554	.002*	0.047	3	0.016	8.699	.000*
TL	0.011	3	0.004	0.236	0.871	0.008	3	0.003	0.507	0.68	0.091	3	0.03	8.937	.000*	0.016	3	0.005	3.035	.046*
Chain	0.003	3	0.001	0.185	0.905	0.005	3	0.002	1.107	0.363	0.251	3	0.084	9.504	.000*	0.006	3	0.002	3.715	.023*
Omn	0.007	3	0.002	0.508	0.68	0.01	3	0.003	1.908	0.151	0.042	3	0.014	8.743	.000*	0.013	3	0.004	3.002	.047*
Can	0.008	3	0.003	1.261	0.307	0.01	3	0.003	1.908	0.151	0.006	3	0.002	0.875	0.466	0.006	3	0.002	2.083	0.125
Path	0.054	3	0.018	3.409	.031*	0.045	3	0.015	4.188	.014*	0.032	3	0.011	2.045	0.13	0.011	3	0.004	1.641	0.202
Clust	0.001	3	0	0.363	0.78	0.001	3	0	0.564	0.643	0.001	3	0	0.315	0.814	0.001	3	0	0.458	0.714
Resource count	526.8	3	175.6	3.875	.020*	213.4	3	71.13	1.462	0.246	373.3	3	124.4	8.83	.000*	119.6	3	39.86	1.664	0.197
Consumer Count	341.6	3	113.9	3.477	.029*	248.3	3	82.78	1.937	0.146	473.6	3	157.9	13.28	.000*	217.8	3	72.61	3.034	.046*