

1 **Negative impact on the reproductive potential of blue jack mackerel *Trachurus***
2 ***picturatus* by *Kudoa* infection of the ovary**

3

4 **Running page head:** *Kudoa* infection of *T. picturatus* ovaries

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1 ABSTRACT

2 Reproduction of *Trachurus picturatus* in western Portuguese coast was studied over one
3 year. During histological analyses, the presence of *Kudoa* sp. was detected on the
4 advanced vitellogenic oocytes. *Kudoa* infections are known to cause economic loss
5 through the induction of post-mortem liquefaction of fish muscles, but ovarian infection
6 as reported in this study, will seriously affect the reproductive potential of the species and
7 thus impact on fisheries productivity. Only females showed gonad infection which led to
8 total degradation of the advance vitellogenic oocytes. No macroscopic nor somatic and
9 condition indices revealed differences between infected and uninfected females,
10 rendering to this infection event an unforeseeable suppression of reproduction.

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12 KEY WORDS: *Kudoa*; stock reproductive potential; suppressed reproduction; ovaries
13 degradation

14

1 **1. INTRODUCTION**

2 Blue jack mackerel, *Trachurus picturatus*, is an oceanic pelagic species found between
3 100 and 575 m depth (Menezes et al. 2006) in the Atlantic Ocean. In the Eastern Atlantic,
4 it occurs from the Bay of Biscay (France) southward to Tristan da Cunha and Gough
5 Islands (Smith-Vaniz 1986). This species is commercially exploited in the southern
6 Northeastern Atlantic. Landings in the mainland Portuguese coast, although highly
7 variable, reached an average value of ca 2800 tonnes/yr in the last decade. Despite its
8 growing economic importance, there is few information on the reproductive strategy of
9 the blue jack mackerel, apart from data on size at maturity and spawning season in the
10 Portuguese continental coast (Costa 2019), Azores (Garcia et al. 2015) and Canary Islands
11 (Jurado-Ruzafa & Santamaría 2013), and the type of fecundity for Madeira Island
12 (Vasconcelos et al. 2017).

13 Traditionally, the reproductive potential has been measured by the spawning stock
14 biomass (SSB), assuming that the number of eggs each female releases is a direct function
15 of its weight and that all eggs have an equal probability of survival (Myers et al. 1994).
16 The term stock reproductive potential (SRP) was later introduced by Trippel (1999) to
17 more accurately represent the annual variation in egg production, being a function of
18 spawners' biomass, sex ratio, maturity at age, realised fecundity and gamete viability.
19 Some of these reproductive attributes are directly related to the fish condition and
20 bioenergetic indices, which depend on diverse variables. Differences in fish condition are
21 often evaluated using the relative liver weight and body condition, since they are related
22 to fecundity and maturation and are easy to quantify (Rideout et al. 2006). A decrease in
23 fish condition is linked to reduced fecundity and skipped spawning (Rideout & Rose 2006;
24 Rodgveller 2019). The present paper reports the infection of large vitellogenic ovaries

1 present in apparent healthy wild fish by the myxozoan *Kudoa* sp, causing a strong
2 decrease of the reproductive potential without a noticeable decrease in the body condition.

3

4 **2. MATERIAL & METHODS**

5 From September 2018 to August 2019, monthly samples of blue jack mackerel were
6 acquired from commercial vessels operating on the west coast of Portugal. Samples were
7 processed in fresh fish, and total length (TL, to the nearest 0.1 cm), eviscerated weight
8 (EW, to the nearest 0.01 g), gonad and liver weights (GW and LW, respectively, to the
9 nearest 0.01 g), and sex were recorded.

10 Gonads were fixed in 10% buffered formalin for posterior histological analysis. For this,
11 a portion of each gonad was dehydrated with ethanol, embedded in methacrylate,
12 sectioned at 3 μ m and stained with toluidine blue. Images were captured with a Leica
13 DFC 290 digital camera attached on a Leica DM 2000 light microscope. Gonads were
14 histologically examined for maturity phases assignment according to the standardized
15 terminology proposed by Brown-Peterson et al. (2011). During histological analyses, the
16 presence of possible *Kudoa* sp. myxosporean parasites, was detected on the advanced
17 vitellogenic oocytes. To confirm the presence of the parasite, a subsample of the same
18 gonads was embedded in paraffin wax and stained with Giemsa.

19 Gonadosomatic index ($GSI = 100 \times GW / EW$), hepatosomatic index ($HSI = 100 \times LW /$
20 EW) and Fulton's condition factor ($K = 100 \times EW / TL^3$) were estimated and significant
21 differences ($\alpha = 0.05$) among infected and uninfected individuals were evaluated with
22 PERMANOVA (Permutational ANOVA, Anderson 2001), under the package vegan 2.5-
23 4 (Oksanen et al. 2014) in RStudio (RStudio Team 2019).

24 The morphology of *Kudoa* sp spores was evaluated with digital photographs and
25 measurements were taken with ImageJ (Schneider et al. 2012). Morphometric characters

1 include spore length, width (W1, W2) and thickness (T1, T2), and length of the large,
2 intermediate and small polar capsules (PCs), measured according to Giulietti et al. (2019).
3 Ratios between spore width 1 and width 2 (W1:W2), thickness 1 and thickness 2 (T1:T2),
4 length of the large PC and length of the intermediate PC (LPCL:IPCL), as well as length
5 of the large PC and the length of the small PC (LPCL:SPCL) were calculated.

6

7 **3. RESULTS**

8 A total of 361 (219 females and 143 males) *T. picturatus* gonads were histologically
9 analysed. Twenty-one females, ranging from 25.2 cm to 45.1 cm, showed advanced
10 vitellogenic oocytes infected by *Kudoa* sp., which showed an intense dark blue in the
11 Giemsa stained sections (Fig. 1). Infected females appeared mainly during and at the end
12 of the reproduction season, from February to August, and for some months represented
13 over 25% of the reproductive females analysed (Table 1). Only female gonads were
14 parasitized with *Kudoa* sp..

15 No differences in the gonad macroscopic features were evident between infected and
16 uninfected spawning capable females (Fig. 2), and significant differences among indices
17 were only noticed for the GSI (GSI: FModel = 2.9950, p = 0.0090; HSI: FModel = 0.7117,
18 p = 0.6298; k: FModel = 0.0927; p = 0.9780) with higher mean values found for infected
19 females in some months (Fig. 3).

20 *Kudoa* sp spores were stellate in shape with 4 unequal pyriform PCs in apical view (Fig.
21 1). The spore and PCs measurements of the present species (mean, SD and range) are
22 given in Table 2.

23 *Kudoa* sp. spores appeared at the periphery of the oocytes, near the zona radiata (Fig. 4a)
24 developing tubular ramified plasmodia from the inner membrane toward the nucleus (Fig.

1 4b). Mass infection of oocytes with only primary growth oocytes remaining in the ovary
2 (Fig. 4c) was observed in several specimens.

3

4 **4. DISCUSSION**

5 Myxozoans of the genus *Kudoa*, with over 100 species described, infect marine and
6 estuarine fish species worldwide (Giulietti et al. 2019). Most of these parasites are
7 described to infect the skeletal musculature, but some occur in the brain, heart, gills,
8 kidney, ovary or intestines (Eiras et al. 2014). Some species are known to cause economic
9 loss through the induction of post-mortem liquefaction of fish muscles and by the
10 production of macroscopic cysts in the fish musculature reducing its market value (Lom
11 & Dyková 2006). The incidence of parasites in fish gonads is difficult to assess since as
12 stressed in Sitjà-Bobadilla (2009) gonads are not always examined in parasite monitoring
13 thus restricting the frequency of myxosporeans described for gonads despite their huge
14 diversity and vast host number. In fact, this author mentions only two myxosporean
15 species specifically affecting the ovary of marine fish, although more species have been
16 described since then. The infection of the ovary as described here, results in a total loss
17 of advanced vitellogenic oocytes leading to a reduction of the species SRP and consequent
18 impacts on fisheries productivity.

19 In the present study no macroscopic evidence of infection was noticed in fresh individuals,
20 with absence of post-mortem liquefaction of infected fish tissues and no visible difference
21 between ovary tissue from infected and uninfected females, a common fact, as gonad
22 infections do not generally produce obvious external signs (Sitjà-Bobadilla 2009).

23 The presence of *Kudoa* infection was only detected in histological slides, after all gonad
24 tissue have been fixed in 10% formalin buffered solution. This inhibited to assess fresh
25 *Kudoa* sp. samples. Few studies report the occurrence of ovary infection (Swearer &

1 Robertson 1999, Mansour et al. 2013, Mansour et al. 2015) and all describe a new *Kudoa*
2 species. Most *Kudoa* species are host specific, infecting fishes of a particular family or
3 even a single species (Whipps & Kent 2006) and many seem to be also tissue specific.
4 *Kudoa nova*, *K. quadratum* and *K. trachuri* were reported infecting *Trachurus* spp
5 musculature (Moran et al. 2009, Campbell 2005, Matsukane et al. 2011), while *K.*
6 *azevedoi* was described in the ovary (Mansour et al. 2013).

7 The *Kudoa* spores found in the present study are of small size which appears to be
8 characteristic of the *Kudoa* species infecting ovaries (Mansour et al. 2013, Mansour et al.
9 2015). However, the fact that measurements in the present study were made on fixed
10 ovary tissue will certainly bias them negatively, hindering a substantiated comparison of
11 the *Kudoa* measurements with those reported by other authors. *Kudoa azevedoi* was
12 described as an ovarian parasite for a close species, *Trachurus trachurus* (Mansour et al.
13 2013), and although there is a possibility for the same species infecting *T. picturatus*,
14 additional studies reporting fresh spores measurements and rDNA sequence analyses will
15 be needed for clarify the species definition.

16 The ovary infection by *Kudoa* sp. detected in several *Trachurus picturatus* specimens is
17 most certainly affecting the SRP on the Portuguese coast. Adlerstein & Dorn (1998) found
18 a significant decrease, as much as 90%, of the average fecundity of *Merluccius productus*
19 infected by *Kudoa paniformis* in the muscle. For gonad infection no quantification on
20 fecundity decrease is available, although such infection will have great impact on fish
21 fecundity (Sitjà-Bobadilla 2009 and references therein).

22 One of the main assumptions in fishery assessments for iteroparous fish species is that
23 after reaching maturity individuals reproduce annually (Macchi et al. 2016). Nevertheless,
24 the failure to spawn in consecutive years (skipped spawning) has been observed in many
25 species (Rideout & Tomkiewicz 2011) and has already started to be incorporated in stock-

1 recruit functions (eg. Brooks 2013). Another suppressed reproduction event is now
2 described, which implies a decrease in the reproductive potential of *T. picturatus*
3 Portuguese population. In this case biological indices do not seem to act as a signal to the
4 reproduction failure, as the affected individuals are only identified by gonad histological
5 examination. An overestimation of annual egg production could occur if such
6 reproductive abnormalities are not taken into account when models are applied. Further
7 studies are necessary in order to clearly quantify reproductive losses and to assess its
8 occurrence in different populations of *T. picturatus*.
9

1 **Acknowledgments**

2 This study was partially supported by the European Maritime and Fisheries Fund
3 MAR2020 project “VALOREJET: Valorização de espécies rejeitadas e de baixo valor
4 comercial”, MAR-01.03.01-FEAMP-0003 and by Fundação para a Ciência e Tecnologia
5 through research contracts attributed to Vera Sequeira (CEECIND/02705/2017) and Ana
6 Rita Vieira (CEECIND/01528/2017) and strategic project UID/MAR/04292/2019.

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12

1 Table 1. Monthly occurrence of *Kudoa* sp. infected females, spawning capable females
 2 (SC females) and total females in *Trachurus picturatus* from west Portuguese coast

3

Month	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Total Females	22	19	15	10	32	26	35	23	27	26	11	15
<i>Kudoa</i> infected females	1	0	1	0	0	2	7	2	2	2	1	3
SC Females	0	0	0	0	17	17	25	4	0	3	1	0

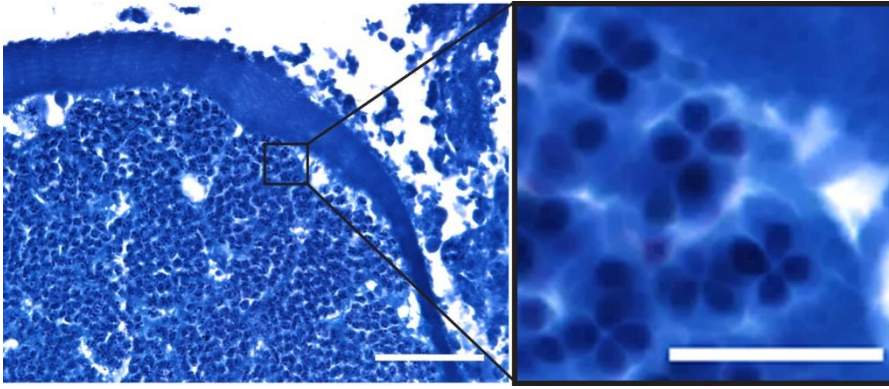
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1 Table 2. Spore morphometry of *Kudoa* sp. found in *Trachurus picturatus* ovary from
 2 Portuguese western coast. Measurements (mean, SD and range, given in μm) were made
 3 on histological slides of formalin fixed ovary tissue. L: spore length (lateral view); W1,
 4 W2: spore width (apical view) T1,T2: spore thickness (apical view); LPCL: length of
 5 large polar capsule; IPCL: combined length of the two intermediate polar capsules; SPCL:
 6 length of the small polar capsule

	Mean	SD	Range
L	3.01	0.42	2.31 – 3.91
W1	4.9	0.55	3.31 – 5.31
W2	4.19	0.63	3.24 – 5.86
T1	3.01	0.44	2.3 – 3.99
T2	2.67	0.32	2.12 – 3.49
LPCL	1.36	0.15	1.09 – 1.80
IPCL	1.21	0.11	1.01 – 1.56
SPCL	1.04	0.11	0.81 – 1.33
W1:W2	1.00	0.09	0.82 – 1.23
T1:T2	1.15	0.10	1.00 – 1.48
LPCL:IPCL	1.13	0.13	0.83 – 1.57
LPCL:SPCL	1.31	0.17	1.03 – 1.78

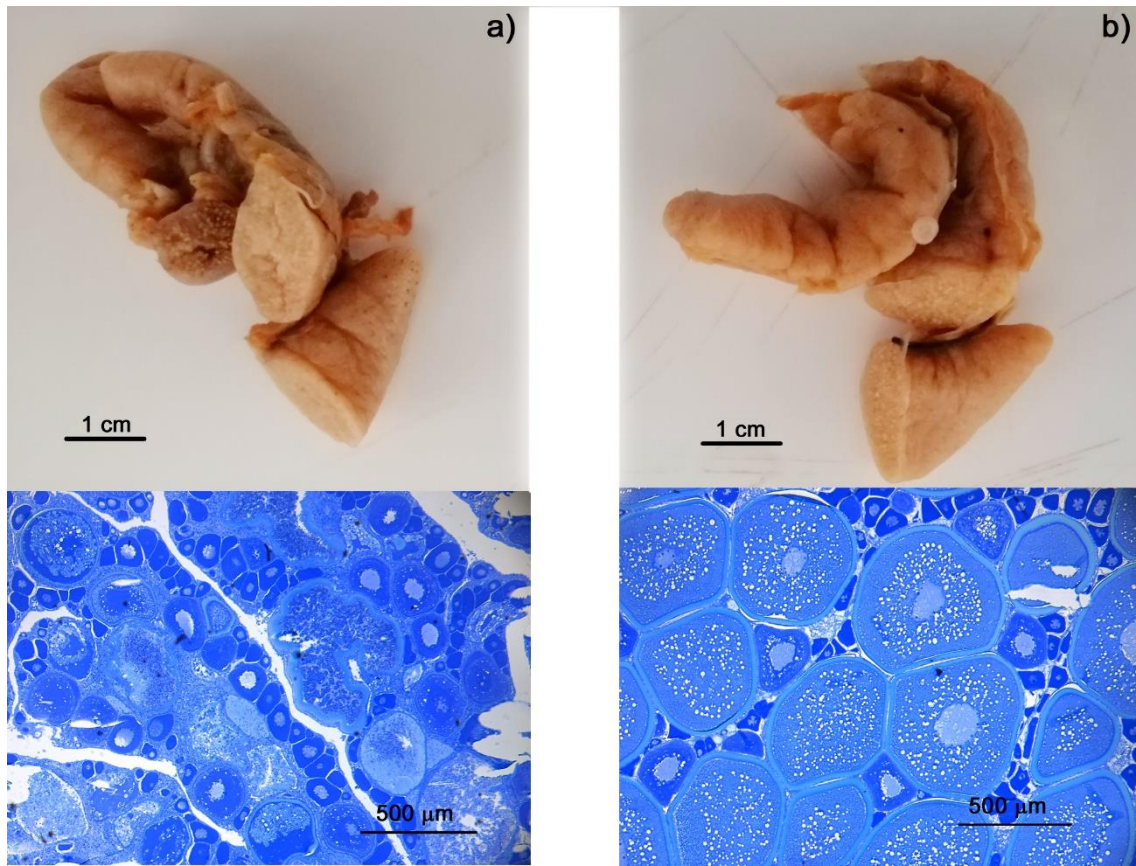
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2 Fig. 1. Giemsa stained histological sections of infected oocyte of *Trachurus picturatus*
3 with a detailed aspect of *Kudoa* spores. Scale bar – 100 μm and 10 μm .

4

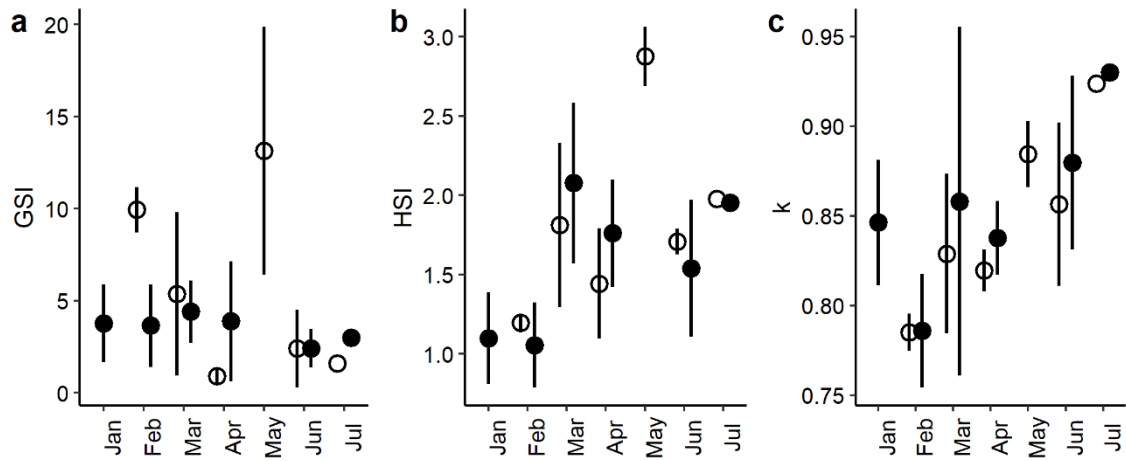


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2 Fig. 2. Macroscopic and microscopic aspect of an infected (a) and an uninfected (b) ovary

3 of *Trachurus picturatus*

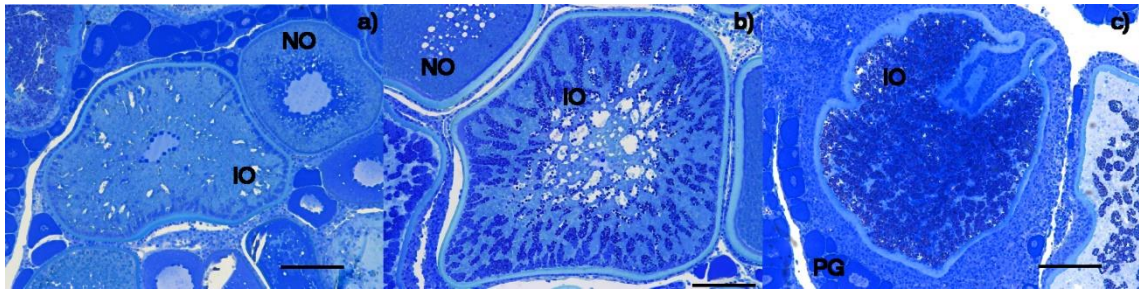
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2 Fig. 3. Mean and standard deviation of a) Gonadosomatic index, b) Hepatosomatic index
 3 and c) Fulton's condition factor for *Trachurus picturatus* females in spawning capable
 4 stage (black dots) and with *Kudoa* sp. infected ovaries (open dots)

5



1

2 Fig. 4. Toluidine blue stained histological sections of infected ovaries of *Trachurus*
3 *picturatus* by *Kudoa* sp. showing infected (IO) and uninfected oocytes (NO). a) *Kudoa*
4 spores condensed at the periphery of an advanced vitellogenic oocyte; b) spore
5 proliferation with yolk fragmentation; c) massive infection with complete degradation of
6 the oocyte. PG – primary growth oocyte; scale bar – 200 μm