Oocyte development in captive Atlantic horse mackerel

Trachurus trachurus

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This 13-month experimental study focused on developing oocyte production in 101 adult female Atlantic horse mackerel. In addition, proxies of energy patterns in relation to oocyte development were investigated. The fish were captured off western Norway and fed to satiation from October 2005 to November 2006 in two replicate adjacent circular tanks. Monthly histological examination of the ovaries indicated that vitellogenic oocytes were present at all times throughout the study period, but that oocyte development did not progress past the incipient migratory nucleus stage. The oocyte diameter (OD) threshold between pre-vitellogenic and vitellogenic oocytes and the mean OD of the leading cohort were investigated, and no hiatus was observed within the OD size distribution around the 185-μm pre-vitellogenic and vitellogenic threshold. Variation in gonadosomatic index, hepatosomatic index, condition factor, and fat content (as measured by the use of a Distell Fish Fatmeter) increased with oocyte development. The observed development of oocyte recruitment and the absence of a hiatus in the oocyte distribution are characteristics of an asynchronous spawner. The species seems to have a prolonged spawning season, judging from the advanced and variety of maturity stages throughout the study period.

Keywords: captive conditions, condition indices, oocyte development, Trachurus trachurus.

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Introduction

The management of Atlantic horse mackerel Trachurus trachurus in the Northeast Atlantic, coordinated by ICES, remains a challenge owing to uncertainty in the assessments (ICES, 2003, 2008). A number of suggestions has been made to improve the assessment, including undertaking egg surveys more frequently, i.e. on a biennial rather than a triennial basis. Currently, the assessment of the different stocks in the area is based primarily on an index of total annual egg production that covers the whole temporal and spatial range of the stock's spawning ground (ICES, 2008). However, application of the method assumes that the relationship between egg production and biomass is constant between assessment years. This assumption has been questioned (ICES, 2003), because there is uncertainty whether horse mackerel is an indeterminate spawner and whether relative fecundity varies interannually (ICES, 2003, 2008). The use of egg production methods (EPMs) for estimating spawning-stock biomass (SSB) is reliant on understanding oocyte recruitment and the fecundity strategy of a species (Jennings et al., 2001; ICES, 2006, 2008; Stratoudakis et al., 2006). Therefore, the use of inappropriate EPMs may lead to poor estimates of SSB and the subsequent stock–recruitment relationship, both of which are necessary for setting biological management reference points.

Horse mackerel in the Aegean Sea (Saronikos Gulf) are considered to have an indeterminate fecundity (Karlou-Riga and Economidis, 1997; Abaunza et al., 2003; Gordo et al., 2008), but there is still debate whether the Atlantic stocks of horse mackerel are determinate or indeterminate spawners (ICES, 2003, 2008). Because of this uncertainty, ICES has not provided SSB estimates for the stock in recent years.

Horse mackerel mature (50%) at 2.5 years of age and 19 cm total length (TL; Jennings et al., 1998). The spawning season appears to be protracted (up to 8 months) across a wide area (Abaunza et al., 2003; Dransfeld et al., 2005), so covering a broad range of environmental conditions. With such a prolonged spawning season, egg production is likely to be regulated by several environmental factors (e.g. temperature and food availability) that could impact on the growth and condition factor of the fish (Rijnsdorp, 1990; Kjesbu et al., 1991; De Oliveira et al., 2006). Such variables may therefore influence spawning activities and the rate of oocyte recruitment (Rinchard and Kestemont, 2003). Knowledge of oocyte development and an understanding of the parameters regulating egg production are necessary for estimating the actual annual egg production and hence the SSB.

Here, we report on oocyte development in Atlantic horse mackerel under captive conditions, so the study provides a further contribution to the study of oocyte development (Macer, 1974; Arruda, 1983; Karlou-Riga and Economidis, 1996, 1997; Gordo et al., 2008), in a species which has previously been shown to have an indeterminate fecundity (Karlou-Riga and Economidis,
1997; Gordo et al., 2008). Compared with field studies, observations in captivity have the advantage of controlled conditions and sample collection. As far as we can ascertain, this is the first report on the dynamics of fecundity development in captive horse mackerel of any species monitored over 13 months. We investigated maturity-stage-specific and month-specific variations in the oocyte size frequency distribution. We also analysed variation in the mean diameter of the leading cohort (LC). Further, we investigated the prevalence of different ovarian development stages and the investment in reproduction by examining various condition indices as a measure of energy reserves. No investigation on fecundity as such was undertaken, because of our conclusions on spawning strategy and oocyte development reported below. Our goal was to develop husbandry techniques that would allow studies on follicle maturation and to provide knowledge that could help to develop tools to determine spawning fraction and batch fecundity (Hunter et al., 1992; Murua et al., 1998). Studies on the reproduction of captive fish provide an opportunity to improve knowledge and understanding of factors that regulate the activity. A particular advantage of working with captive populations is that sampling can be from a single school or a population, so a coherent picture of ovary development can be built up over many months.

Material and methods
Collection and rearing of fish
The study was conducted at the Institute of Marine Research, Matre Research Station, between October 2005 and October 2006. In September 2005, 600 male and female fish were caught by purse-seine in outer Mavjord, close to the research station, and transported to the station using a well-boat. These fish were randomly assigned (300 in each) to two 5-m circular tanks. The average body weight was 530 g and the fish were fed to satiation three times a week. Mortality was negligible. The temperature regimes, which were natural water temperature regimes of the station-water source, were the same in both tanks (Figure 1). The fish were reared indoors, under a natural photoperiod, with light entering through windows in the roof above the tanks. All artificial lighting within the experiment hall was programmed to be off during the presumed spawning period, no stress was applied, and batch fecundity was followed for histological analysis. Histology preparations were undertaken using standard techniques and products based on methacrylate (currently Technovit® 7100) as an embedding medium (Greer Walker et al., 1994). Several histological sections of 4-μm thickness were prepared from each ovary and stained with 2% toluidine blue and 1% borax. Structures such as the nucelus, the yolk granule, and the chorion were stained to different degrees of blue. From these sections, oocyte development stage (maturity stage) was classified microscopically, based upon the developmental stage of the most advanced oocytes (West, 1990), as described by Fonn et al. (2007), specifically using a horse mackerel maturity stage key (Abanuzza et al., 2003). Pictures of the histological sections were digitized using an Olympus image-analysis system mounted on a microscope and used for maturity staging, as well as for observations of atresia and spawning markers (post-ovulatary follicles, POFs).

Oocyte development
The process of oocyte development pattern was investigated by:
(i) analysing oocyte diameter (OD) progression at different maturity stages, and (ii) analysing monthly OD evolution for all 101 fish. OD was determined using Image J and the method of Thorsen and Kjesbu (2001). All measurements were made on whole-mount sample materials. Advanced stages (vitellogenic stages) were measured automatically (Thorsen and Kjesbu, 2001), but all oocytes that did not fall within the parameter set (the smaller oocytes) were measured manually (Thorsen and Kjesbu, 2001). The same manual measuring procedure was used for less-developed oocytes (pre-vitellogenic stages). On average, 150 oocytes were measured per fish. The mean size of the LC in every fish was then determined from the oocytes measured and defined as the largest 10% of those oocytes from that fish (Kjesbu, 1994; Thorsen and Kjesbu, 2001). To investigate oocyte composition and size frequency, a further 300–500 oocytes per fish per development stage were measured.

Investigation of the energy pattern
To investigate the causes of variation in oocyte development and surplus energy allocation, several variables were considered as proxies of reproductive potential and investment. The gonadosomatic index (GSI), GSI = 100 × Wg/Wb, the hepatosomatic index (HSI), HSI = 100 × Wh/Wb, and the condition factor (K), K = 100 × Wb/TL3, were calculated. The fat content of the fish body was measured with a Distell Fatmeter, Model FM692, using the instrument’s calibrated setting for horse mackerel. More specifically, we used the setting specifically designed to represent the fat content of the whole fish carcass through the skin. The instrument on this setting gives an accuracy of ±1% for fish with 2–15% fat, ±2% accuracy for 16–30% fat, and ±4% accuracy for fat content of 31% and more (manufacturer’s specifications).
Results
The adult female horse mackerel used in the experiment were on average 38 cm (± 2.2 s.d.), 596 g (± 141 s.d.), and all >12 years old, with most of them older than 15 years. Although their previous spawning history was not known, at that size and age, they were all regarded as physiologically mature (Abauanza et al., 2003). At the completion of the experiment, there were no significant differences between the tanks in female fish length (p = 0.22) or weight (p = 0.19), nor in male fish length (p = 0.75) or weight (p = 0.07) (Student’s t-test). As there were no significant tank effects, the two datasets were pooled for analysis.

The water temperature was the same in both tanks and ranged between 8 and 13°C during the period of sampling (Figure 1), water being warmer in late summer from August on, and coolest between April and May, just as the ovaries with migratory nuclei were most abundant in our samples. Oocytes developed gradually, but did not progress past the migratory nucleus (incipient) stage (Figure 2). The four maturity stages observed were represented in the samples in different proportions throughout the study period (Figure 3). In all ovaries, primary oocytes were present and dominated in October (80%) and November 2005 (80%), and again in October 2006 (60%). The LC OD in females with the most advanced maturation increased rapidly from January to February (Figure 4), with an increase in the number of fish with more developed stages towards March and April, as seen by the decrease in the proportion of fish with primary oocytes and an increase in the proportion of those in the migratory nucleus stage in April (Figure 3).

The mean ODs of individual fish increased with maturity stage (Figure 5). When only LC ODs were analysed, there were no significant differences between primary and yolk vesicle oocytes (Student’s t-test, p = 0.27), but the difference was significant between yolk vesicle and yolk granule oocytes (p < 0.00), and between yolk granule and migratory nucleus oocytes (p < 0.00). Progression of the same LC per month indicated a gradual increase from October 2005 (144 µm ± 7 s.e.) to May 2006 (359 µm ± 71 s.e.), then a decrease to October 2006 (246 µm ± 84 s.e.; Figure 4), but not as low as in October 2005 because there were more fish in the yolk granule stage in October 2006 than in October 2005. In the most advanced case (migratory nucleus), the follicle frequency distribution showed the accumulation of migratory nuclei stage follicles (Figure 5), suggesting that those fish could have ovulated within a short time if they received the right cue. No eggs were observed in the fitted egg collectors, nor were there POFs in the histological slides. Although not quantified, atresia was observed in the advanced development stages during the final months of the experiment, but not at the same stages during the earlier months.

An examination of the pattern of energy reserve proxies indicated an increase in GSI, HSI, K, and fat content, with advancement in oocyte development (Table 1). GSI increased significantly in conjunction with development from primary to migratory nucleus oocytes (Student’s t-test, p = 0.01), and also with development from yolk vesicle to migratory nucleus oocytes (p = 0.03). HSI differences were only significant between primary and yolk granule oocytes (p = 0.02), whereas differences in K were significant between primary oocytes and yolk granule oocytes (p < 0.00), and between primary oocytes and migratory nucleus oocytes (p = 0.01). Fat content also increased significantly with development between primary and yolk granule oocytes (p < 0.00), and between yolk vesicle and yolk granule oocytes (p = 0.02).

Discussion
An unequivocal determination of the mechanisms of oocyte production and hence egg production of horse mackerel is crucial to estimating fecundity and subsequent egg production, and hence is vital for stock assessment, forming the cornerstone of setting biological reference points for management. This novel study on captive horse mackerel used protocols similar to those laid out in Greer Walker et al. (1994) for mackerel (Scomber scombrus), to assess the oocyte development strategy in horse mackerel. There was a continuous size distribution of oocytes in ovaries of individual fish, with no hiatus at the
pre-vitellogenic and vitellogenic threshold of females with oocytes at various advanced development stages throughout the study period. These are characteristics of asynchronous oocyte development (Macer, 1974; Abaunza et al., 2003). We also observed a range and overlap in the mean diameter of advanced oocytes (LC) within and between maturity stages, which demonstrates the presence of various types of oocyte at similar ODs. These observations support the conclusions of other authors on other stocks of horse mackerel from the Saronikos Gulf (Greece), Northeast Atlantic generally, and the Mediterranean (Karlou-Riga and Economidis, 1997; Gordo et al., 2008), that horse mackerel can be considered to have indeterminate fecundity. The absence of a hiatus at 185 μm (Withthames and Greenwood, 2002), the threshold between pre-vitellogenic (primary oocytes) and vitellogenic (yolk vesicle, yolk granule, and migratory nucleus stages), supports the idea that the species has asynchronous oocyte development. Support for this observation comes from a substantial overlap in oocyte size distribution for pre-vitellogenic and vitellogenic stages. Further support came in the form of the observed continuous oocyte size distribution and the presence of oocytes at different development stages in one ovary.

Figure 2. (a–d) Whole mount and (e–h) photomicrographs of sections of ovaries from captive female horse mackerel T. trachurus at different maturity stages of development: (a and e) primary oocytes, (b and f) yolk vesicle (cortical alveoli) oocytes, (c and g) yolk granule oocytes, and (d and h) migratory nucleus oocytes. nu, nucleus; n, nucleoli; ca, cortical alveoli (yolk vesicle); yg, yolk granule.
LC oocyte development over time was most advanced in April and May, but with considerable variation in average LC size, because some fish had not reached vitellogenesis, so their LC comprised smaller oocytes. Meanwhile, the broader spread in LC diameter at the yolk granule stage, which is significantly different from the development stages before and after, suggests that early vitellogenic oocytes vary considerably in size and that the currently used threshold of 185 \( \mu \text{m} \) (Witthames and Greenwood, 2002) could lead to underestimation of the fraction that is sexually mature. Our mean LC diameter of vitellogenic oocytes was in the same range and at the same time as observed in the wild (ICES, 2008), suggesting that the laboratory results are indicative of the situation in the field. However, oocyte development in this study did not progress beyond the migratory nucleus (incipient spawning) stage even after a year of excess feeding. This suspension of oocyte development indicates that necessary stimuli and cues were inappropriate. It is also an example of skipped spawning type 3, in which follicles grow to the point of final maturation but then are re-absorbed without undergoing ovulation to release the egg (Rideout et al., 2005). We speculate that temperature, photoperiod, and perhaps the depth of water in the tanks and/or illumination levels could be outside the required physiological response ranges, because such factors are important for other teleosts (Lake, 1967; Mackay, 1973). In addition, changes in food availability can stimulate oocyte development, as demonstrated with golden perch (Macquaria ambigua; Collins and Anderson, 1999). However, a prime candidate might be temperature, because the temperature of the experimental tanks was in the range 8–10 \( \degree \text{C} \) most of the time, and fell to its lowest levels of 7–8 \( \degree \text{C} \) when fish with a migratory nucleus LC were most abundant. This temperature range is well below the preferred temperature reported for spawning in wild populations of horse mackerel, reported as 12–14 \( \degree \text{C} \) during the spawning season (Coombs et al., 2001).

Our seasonal variation in the proportion of fish at different oocyte development stages (maturity stages) indicates that spawning time would be prolonged. Although the number of females sampled per month (ten fish) was relatively small, there were clear variations in gonad development over time. The presence of various maturity stages over the 13 sampling months can be explained in two ways. First, perhaps environmental conditions in captivity (temperature, light conditions, food availability) might not have differed significantly enough to act as a reproductive stimulus to all fish at the same time (Lake, 1967). Second, it could be the result of the protracted spawning season of horse mackerel observed in the wild (Macer, 1974; Solá et al., 1990; Abaunza et al., 2003).

The observed decline in OD and the absence of advanced maturity stages as well as a large proportion of the primary oocyte stage in August and October suggests that after advancement to a prespawning stage, advanced oocytes are re-absorbed and that the fish return again to a less advanced maturity stage.
probably as the new batch cycle starts. Atresia was found in the advanced development stages during the final months of the experiment, but not at the same stages during earlier months. This suggests that the process of atresia could be completed in a short period, so that there might be no difference between early stage ovaries of prespawning fish and the same stages observed during or after the spawning season.

We found a positive correlation between energy reserve proxies and oocyte development and average OD. This implies that oocyte maturation is not reached at the expense of either body muscle or liver lipids, but rather that maturation is reached with energy directly from food and not from stored reserves. This was similar to the observation made by Hunter and Leong (1981) for northern anchovy (Engraulis mordax). This is to some extent not surprising because horse mackerel feed throughout their entire spawning period and could behave as income breeders (Bonnet et al., 1998). However, it is not clear from this study what the quantitative role is, but it is likely that the energy used for oocyte development was not from the energy reserves, probably because food was abundant throughout the experiment. However, because the fish did not mature to a more advanced stage where more investment in oocyte maturation could have been evident (Macer, 1974), it might be that a different situation would be observed as oocyte development approaches immediate prespawning stages (oocyte hydration).

Our observations of the development of oocytes and the absence of a hiatus in the oocyte size distribution in this study are indicative of an asynchronous indeterminate spawner, one likely to have a prolonged spawning season with various maturity stages within and between fish over a period. We have

Table 1. Proxies of energy reserves (GSI, HSI, K, and fat content of captive Atlantic horse mackerel T. trachurus) at different maturity stages.

<table>
<thead>
<tr>
<th>Oocyte development stage</th>
<th>n</th>
<th>GSI Mean ± s.d.</th>
<th>HSI Mean ± s.d.</th>
<th>K Mean ± s.d.</th>
<th>Fat content Mean ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary oocyte</td>
<td>31</td>
<td>1.73 ± 0.97</td>
<td>1.25 ± 0.62</td>
<td>0.98 ± 0.17</td>
<td>17.54 ± 6.44</td>
</tr>
<tr>
<td>Yolk vesicle oocyte</td>
<td>31</td>
<td>1.89 ± 0.91</td>
<td>1.56 ± 0.82</td>
<td>1.07 ± 0.23</td>
<td>19.63 ± 10.17</td>
</tr>
<tr>
<td>Yolk granule oocyte</td>
<td>32</td>
<td>2.35 ± 1.01</td>
<td>1.70 ± 0.70</td>
<td>1.19 ± 0.22</td>
<td>25.94 ± 28.35</td>
</tr>
<tr>
<td>Migratory nucleus oocyte</td>
<td>7</td>
<td>4.25 ± 2.34</td>
<td>1.72 ± 0.95</td>
<td>1.27 ± 0.14</td>
<td>28.37 ± 8.76</td>
</tr>
</tbody>
</table>

Figure 5. Size frequency distribution of OD of captive horse mackerel T. trachurus (bars) through subsequent maturity stages. The four panels represent oocytes of four fish measured from fixed whole-mount ovary material. The vertical line indicates the mean of all recordings of individual female LC diameter, and the horizontal line represents the interquartile range of each development stage.
demonstrated for the first time that horse mackerel thrive in captivity (i.e. take food willingly, appear to remain healthy over a long period, and have a low rate of mortality). However, in further experiments, we need to ensure that environmental conditions (especially water temperature) during the spawning period accurately reflect conditions in the field that likely initiate oocyte final maturation and hence spawning.

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