Female Reproductive Biology of the Klunzinger's Mullet (Liza klunzingeri) in the Persian Gulf and the Oman Sea

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Abstract
Histological changes and maturity stages during the reproductive cycle of the ovaries of Klunzinger's Mullet (Liza klunzingeri) were investigated. The evaluation of the spawning season was carried out based on monthly samples from 3 stations in the Persian Gulf and the Oman Sea. Sex ratio was approximately 1:3 (M: F). The maturity stages of female L. Klunzingeri are: Virgin, Developing Virgin, Developing, Developed, Gravid and Spent. Observations on seasonal maturity stages indicated that the species had a spawning period from November to March. The highest and the lowest gonadosomatic index (GSI) of 6.03 and 0.28 occurred in December and May, respectively. Individuals reached 50% maturity at 15.4 cm Total Length (TL). Histological analyses indicated group synchronous ovarian development occurred in L. klunzingeri.

Keywords: Mullet, Liza klunzingeri, Gonadosomatic Index, Length at sexual maturity, Sex ratio, Persian Gulf

1. Introduction

The mullets are a family (Mugilidae) of ray-finned fish found worldwide in coastal tropical waters. Klunzinger's Mullet (Liza klunzingeri) is an important resource in the Persian Gulf and the Oman Sea. In Hormozgan province, the total catch was 220 tons in the 2007-2008 fishing season. L. klunzingeri, formerly known as L. carinata (Carpenter et al., 1997) is caught commercially using beach seines and stake traps (Abou-Seedo and Dadzie, 2004). Studies of the reproductive biology and histological examination of gonads are appropriate to determine the precise spawning period and frequency in a breeding season. Many freshwater fish species in the subtropical or tropical waters have long spawning seasons, whereas those species that inhabit temperate waters, where conditions in winter are more severe, typically have shorter and more defined breeding periods (Conover, 1992).

The multispecies fishery in the Persian Gulf is dominated by many commercially important species including, klunzingeri, Pampus argenteus, Acanthopagrus spp., Epinephelus tauvina, Formia niger, Tenualosa ilisha, Pomadasys kaanan, Otolithes argenteus and O. ruber (Al Husaini et al., 2001; Al-Husaini, 2002; Bishop, 2002, 2003).
Numerous investigations have been conducted on Mullet species (Liu et al., 2009; Lee et al., 2009; Maggio et al., 2009; Azad et al., 2008; Bu-Olayan et al., 2005; Dadzie et al., 2005; Evans et al., 2004; Evans et al., 2002; Abou-Seedo et al., 2002), but there is very limited documentation on ovarian development of this species in the Persian Gulf and the Oman Sea.

Objectives of this study were to investigate the spawning season duration and size at maturity and to increase knowledge of the reproductive biology of *L. klunzingeri* in the coastal waters of the Hormozgan Province, Iran.

2. Material and Methods

A total of 403 individuals of *L. klunzingeri*, ranging from 10.0 to 18.3 cm total length were sampled monthly from the Persian Gulf and the Oman Sea between October 2007 to September 2008 (Fig. 1).

Fish were caught using beach seines and stake traps and were collected regularly and directly from artisanal fishermen at three landing sites at Bandar Abbas, Jask and Salakh (Fig.1). Samples were transferred immediately to the laboratory where fork length (to the nearest cm) and body weight (to the nearest 0.01 kg) of each fish were recorded. The fishes were then dissected and the gonads excised and weighed (0.1 g) for the evaluation of the spawning season. Thirty fish were randomly selected each month of the study. The stages of gonad maturity were determined following the methods outlined by Biswas (1993) and Busacker et al. (1990). A section of female gonad was fixed in Bouin’s fixative and then processed histologically to track gonad development within the gonad lobe. Transverse sections (5-7 μm) were stained with haematoxylin and eosin.

Monthly changes in three variables were analyzed to determine the spawning season of *L. klunzingeri* (West, 1990):

1) Gonadosomatic index (GSI)-an indirect method for estimating spawning season of a species (Biswas, 1993)-was calculated using equation 1:

\[
\text{GSI}= \frac{W_G}{W_{\text{Total}}} \times 100
\]  

where \(W_G\) is Gonad weight and \(W_{\text{Total}}\) is Total body weight.

2) Hepatosomatic index (HSI): an indirect method for estimating spawning season of a species (Biswas, 1993)- was calculated using equation 2:

\[
\text{HSI}= \frac{W_L}{W_{\text{Total}}} \times 100
\]  

Where \(W_L\) is Liver weight and \(W_{\text{Total}}\) is Total body weight.

3) Length at sexual maturity: the proportion of specimens within each centimeter size class that had gonads in designated stages of maturity was calculated. As per the method of Pope et al. (1983), the length at first spawning (L50), represented the length at which 50% of the population were mature and the designated stages 3–5, were determined using equation 3:

\[
P = \frac{100}{1 + \exp (a + bTL)}
\]  

where \(P\) is percentage of mature individuals as a function of size class (TL); and \(a\) and \(b\) are specific parameters that change during the life cycle.

Maturity stages of the ovary were determined using methodology of Makeeva (1992) and based on macroscopic and microscopic observations.
3. Results

3.1. Maturity stages

There were six maturity stages as follows:

Stage I (Virgin): The ovary is small, thread like, transparent in color and occupies about 10% of the peritoneal cavity. Sex cells are not discernible macroscopically (Fig. 2A). Microscopic examination reveals oogonia, early perinucleolar oocytes and few late perinucleolar oocytes. The centrally-located nucleus has a conspicuous nuclear membrane. This stage is characterized by the presence of a zone of strongly basophilic cytoplasm (Fig. 3A).

Stage II (Developing virgin): Oocytes are slightly larger, pink in color and translucent. The ovary occupies about 25% of the body cavity. The eggs become visible (Fig. 2B). Microscopically, oogonia continue to be present. However, the majority of the germ cells are in the primary growth phase, with an increasing number of large- size perinucleolar stage oocytes (Fig. 3B).

Stage III (Developing): The ovary enlarges and is larger than that in previous stages. It is reddish-yellow in color and occupies about half of the body cavity. Many blood capillaries are visible around the organ (Fig. 2C). Microscopically, lipid vesicle stage I (vitellogenic) oocytes become visible (Fig. 3C).

Stage IV (Developed): The ovary is swollen, reddish-yellow in color, and occupies about two thirds of the body cavity. Eggs are highly visible and numerous (Fig. 2D). Histological examination reveals numerous large, advanced vitellogenic oocytes with large lipid vesicles and yolk granules. Few primary growth phase oocytes are present. This stage is characterized by the presence of a zone of strongly acidophilic cytoplasm. The walls of oocytes increase in thickness (Fig. 3D).

Stage V (Gravid): The ovary is maximally distended, yellow in color, and completely fills the body cavity. Large vitellogenic oocytes are clearly visible beneath a thin, almost transparent ovary wall, and are easily extruded with slight pressure on the belly of animals (Fig. 2E). Histologically, large size oocytes with coarse yolk granules scattered in the cytoplasm, are present (Fig. 3E).

Stage VI (Spent): This stage was not observed in the samples collected for this study.
3.2. Seasonal Distribution of Maturity Stages:

Gonad development frequency is shown in Fig. 4. In this study, gonads were largely inactive from April to August as seen by the large proportion in the Development stage III.

Gametogenetic activity commenced in September increasing to November when some spawning activity was observed (Stage V germ cells). Spawning activity continued until March. Based on GSI values this study found that the spawning season of *L. klunzingeri* in the Hormozgan province extended from November to March.

3.3. Gonadosomatic Index

A monthly variation in GSI is evident as shown in Fig. 5. The highest GSI was found in December with a gradual decrease to the lowest level in May and clearly indicated that the species spawned in winter.

3.4. Hepatosomatic Index

HSI findings (Fig. 5) fluctuate during the annual cycle. Liver activity increased in the months of May/June and September but decreased during the
spawning season.

Fig. 6 shows that 50% of females reached maturity at a total body length of 15.4 cm.

Fig. 6: Logistic curve fitted to the proportion of mature females of *L. klunzingeri* (maturity stages 3–5) in relation to length.

3.5. Sex ratio

Of the 403 specimens sampled, 299 were females and 104 were males, a sex ratio of 1:2.87, respectively. (Table 1).

4. Discussion

The histological results strongly suggested that the spawning season of *L. klunzingeri* extended from November to March forming a peak in December in coastal waters of the Hormozgan province. These findings are consistent with those reported by Valinassab (2006) in the coastal waters of Khuzestan province and Ismail et al. (1998) for Kuwaiti waters, but differ slightly to those reported for Kuwaiti waters (Abou-Seedo and Al-Khatib, 1995). In this study, the reproductive cycle commenced in May, culminated in spawning from December to February. In a further study by Ismail et al. during 1998-99, gametogenesis commenced in October and spawning started in November finishing in March. In the Karachi- Sind waters of Pakistan, Hoda and Qureshi (1989) observed a spawning period from October to March in the same species.

Slight variation in spawning time in different regions are primarily because of differing environmental parameters, such as temperature, light and salinity which cause changes in physiological activities and consequently spawning time (King, 1995).

The histological studies demonstrated that the process of development of the *L. klunzingeri* did not differ markedly from that described by Abou-Seedo et al. (1995) for the same species.

Table 1. Monthly sex ratio in captured of *L. klunzingeri* during the study period in Iranian waters

<table>
<thead>
<tr>
<th>Month</th>
<th>Sample observed</th>
<th>Number of males</th>
<th>Number of females</th>
<th>Length range of males</th>
<th>Length range of females</th>
<th>Sex ratio M:F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct-07</td>
<td>38</td>
<td>8</td>
<td>30</td>
<td>13-15.30</td>
<td>12.20-16</td>
<td>1:0.26</td>
</tr>
<tr>
<td>Nov</td>
<td>58</td>
<td>24</td>
<td>34</td>
<td>11.5-15.60</td>
<td>10.4-16</td>
<td>1:1.41</td>
</tr>
<tr>
<td>Dec</td>
<td>54</td>
<td>24</td>
<td>30</td>
<td>12.7-14.30</td>
<td>12.9-14.4</td>
<td>1:1.25</td>
</tr>
<tr>
<td>Jan-08</td>
<td>31</td>
<td>6</td>
<td>25</td>
<td>10-13.10</td>
<td>11.7-17.6</td>
<td>1:4.16</td>
</tr>
<tr>
<td>Feb</td>
<td>33</td>
<td>4</td>
<td>29</td>
<td>12.2-15.60</td>
<td>10.7-17</td>
<td>1:7.25</td>
</tr>
<tr>
<td>Mar</td>
<td>26</td>
<td>18</td>
<td>8</td>
<td>12.7-14.50</td>
<td>13-15.2</td>
<td>1:0.44</td>
</tr>
<tr>
<td>Apr</td>
<td>30</td>
<td>5</td>
<td>25</td>
<td>13-14</td>
<td>12.9-15.5</td>
<td>1:5</td>
</tr>
<tr>
<td>May</td>
<td>21</td>
<td>4</td>
<td>17</td>
<td>14.5-15</td>
<td>12.6-15.5</td>
<td>1:4.25</td>
</tr>
<tr>
<td>Jun</td>
<td>24</td>
<td>8</td>
<td>16</td>
<td>12.2-13.6</td>
<td>12.5-14.6</td>
<td>1:2</td>
</tr>
<tr>
<td>Jul</td>
<td>29</td>
<td>0</td>
<td>29</td>
<td>0</td>
<td>12.7-17.4</td>
<td>0:29</td>
</tr>
<tr>
<td>Aug</td>
<td>29</td>
<td>0</td>
<td>29</td>
<td>0</td>
<td>13.8-16</td>
<td>0:29</td>
</tr>
<tr>
<td>Sep</td>
<td>30</td>
<td>3</td>
<td>27</td>
<td>13.8-16</td>
<td>13.3-18.3</td>
<td>1:9</td>
</tr>
<tr>
<td>Total</td>
<td>403</td>
<td>104</td>
<td>299</td>
<td>10-16</td>
<td>10.4-18.3</td>
<td>1:2.87</td>
</tr>
</tbody>
</table>
The keeled mullet shows synchronous ovarian development culminating in spawning over a 5 month period. The cycle of maturation and depletion of gonads occurs only once throughout the year. The gonads show a regular seasonal change and at any given time, the state of maturity is approximately uniform in all individuals, although there are many large and small eggs in some ovaries at the same time. This does not indicate partial spawning in this species, because the small eggs remain in ovary and are gradually reabsorbed (Nikolsky, 1963). Ovary synchrony in this species has also been reported from Kuwaiti waters (Abou-Seedo et al., 2004) and in coastal waters of Khuzestan province (Valinassab 2006). The relationship between the proportion of mature females and fish size, fitted by means of the logistic curve, indicates that many animals large enough to spawn probably did not spawn in the current season (La Mesa, 2003). The first sexual maturation is an important point in the animal’s life history and must be taken into account for successful fish management. This study shows that 50% of females reach maturity at a total body length of 15.4 cm (TL). These results compare well with those reported by Abou-seedo and Dadzie (2004). In their study, females reached maximum reproductive capacity at 14.1-18 cm total length. However, Valinassab et al. (2006) found females reached a maximum reproductive capacity at 17.6 cm (FL) in Khuzestan waters. This shows that the stocks of *L. klunzingeri* in Hormozgan province and Khuzestan Province probably are different.

In this study, the sex ratio determined from limited data was approx. 1:3 male: female. A male: female sex ratio of 1.5:1 is reported by Ismail et al. (1998), 1:2 by Abou-Seedo and Dadzie (2004) and 1:5 by Valinasab (2006) for the same species. This could be due to different fishing factors related to seasons and schooling in feeding and spawning grounds (Abou-Seedo and Dadzie, 2004). Abou-Seedo et al. (2004) also found that HSI reduced in the spawning period. They inferred that this was due to the fact that animals stopped feeding during this time and used energy stored by the liver for reproductive activity resulting in lower HIS values. Further study is needed to clarify whether feeding stops for this species in Hormozgen province waters during the spawning season.

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