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Ovarian Development and Sexual Maturation of Female Striped Catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) Reared in Captivity

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Abstract

Developmental changes in the ovary of *Pangasianodon hypophthalmus* (Sauvage, 1878) (initial weight 284±24.2 g; initial age 6 months) held in captivity were examined over an 8-month period. Ovarian development was assessed macroscopically and microscopically, and histological analysis was used to establish the stages of oocyte development. Oocyte development could be divided into eight stages, but oogonia and perinucleolar oocytes, considered as part of the initial growth phase, were observed in ovaries throughout the study period. The presence of more than two developmental stages of oocytes in a single ovary throughout the study period indicated that *P. hypophthalmus* possesses asynchronous ovaries. Sexual maturation was recorded in 13-14 month old females, when mean length was ≥ 46.99 ±1.04 cm, and body weight had reached ≥ 989.70±59.98 g. This indicates that the time required for *P. hypophthalmus* to attain sexual maturity is shorter in captivity than in the wild.

Introduction

The striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) is an important freshwater aquaculture species in South and South East Asia, with significant production taking place in Vietnam (Bui et al. 2010), Malaysia (Asdari et al. 2010) and Bangladesh (Ahmed and Hasan 2007). Despite this, information about ovarian development and reproductive biology of farmed *P. hypophthalmus* is scarce, even though knowledge about reproductive processes is required in order to control the timing of sexual maturation and spawning, and to improve gamete quality and reduce production costs of farmed fish (Coward et al. 2002; Mylonas and Zohar 2007; Shafiei et al. 2010; Adebiyi et al. 2011). Fish reproduction may be influenced by many factors, including seasonal changes in photoperiod, rainfall and water temperature, along with physico-chemical features of the water body and features relating to holding conditions (Bromage et al. 2001; Glasser et al. 2004; Dorostghoal et al. 2009).

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In the case of *P. hypophthalmus*, Potaros and Sitasit (1976) mentioned that the spawning season was between June and September in Thailand, while Saidin and Othman (1986) reported that broodstock were in reproductive condition from June to January in Malaysian earthen ponds. In Indonesia, 3-4 year old *P. hypophthalmus,* weighing 2-5 kg, were found to be suitable for use as broodstock (Legendre et al. 2000), while best spawning performance was observed in hatcheries of the Mekong Delta, Vietnam when broodstock were 5.7±0.5 kg (Bui et al. 2010).

An understanding of the dynamics of the reproductive events that occur during oocyte development is needed to obtain information about the size and age at maturity of female fish, as well as to gain knowledge about the duration of the spawning season and spawning patterns (Coward and Bromage 1998; Bromage et al. 2001; Shabanipour and Hossayni 2010). Histological analysis is a useful, and often used, tool employed to study ovarian development and in the definition of gonad maturity stages in fish (West 1990; Mendonca et al. 2006; Babin et al. 2007; Shinkafi et al. 2011).

The main aim of this study was to use oocyte stage development to determine the timing of sexual maturation in female *P. hypophthalmus* raised in captivity. To this end monthly sampling of immature fish was undertaken from the time they were 6 months old, and oocyte development was examined by considering morphological changes and histological analysis of the ovary.

Materials and Methods

Fish and rearing conditions

One thousand two hundred, 6-month old striped catfish *P. hypophthalmus* (weight 284 ±24.2 g) were randomly distributed into 10 outdoor canvas tanks, with 120 fish in each tank to give a stocking density of 30 fish·m^{-3}. Each tank, which measured 4 m×1 m×1 m (length × width × height), was established within a flow-through system with aeration, with water being supplied from an overhead tank containing de-chlorinated water. During the experiment, dissolved oxygen was 5.10 ±1.2 mg·L^{-1}, pH was 6.29-6.90 and water temperature was 29±2 ^{0}C. Tanks were cleaned monthly and about half of the water in the system was drained out to reduce the accumulation of sediment and nitrogenous waste. The tanks were covered with netting to prevent escape of the fish. The fish were fed with floating commercial diet (protein 32%, lipid 10%, fibre 6%, Cargill Feed Sdn. Bhd, Malaysia) at 2-3% body weight (BW), twice a day, at 09:00 and 17:00.

Ovary sampling and analytical protocol

Between10-16 female fish were sampled monthly over the 8-month period from May-December, 2010 (Table 1). The number of females sampled each month differed because the fish were held in mixed–sex groups and in the initial stages of the study when the fish were small, identification of the sex of individuals based on external appearance was not possible.

Fish were selected at random and anaesthetised with Aquadine (Fish Stabilizer, International Fish S.O.S Assoc.). The body weight (BW) and total length (TL) were recorded to the nearest 0.01 g and 0.01 cm respectively. Female fish were then dissected to obtain the ovaries and livers which were then weighed separately and used for the determination of the gonadosomatic index (GSI) and the hepatosomatic index (HSI) using the following formulae:

Gonadosomatic index = Gonad weight/body weight*100 (Dorostghoal et al. 2009)

Hepatosomatic index = Liver weight/body weight*100 (Cek and Yilmaz 2009; Reidel et al. 2010).

Physical examination of the genital papilla was carried out and macroscopic appearance of the ovary was observed. For histology studies, transverse sections of ovarian samples were taken from the anterior, middle and posterior parts of the right and left ovaries of each fish, and these sections were fixed in 10% formalin solution for 24 hr before processing. The ovary tissue sections were then transferred to 50% ethanol and were processed using routine histological protocols (Davenport 1969), tissues were embedded in paraffin blocks and sectioned at 8 µm thickness.

The thin sections were mounted on glass slides with Pertex (Merck Ltd, U.K) and stained using haematoxylin and eosin (H & E). The slides were observed with a light microscope and images were computer digitised using an image capture analysis system (Image Cell A, Stereomicroscope, Camera-Xcam-α Model, Olympus SZX9ICAS, Japan) to determine the histological stage development of ovarian oocyte. Histological classification and monthly oocyte stage development pattern was based on the maximum number of advanced oocytes present in the sections (West 1990; Coward and Bromage 1998; Trip et al. 2011). The maturity stages of the *P. hypophthalmus* ovary were determined using the modified gonad maturity scale developed by Estay et al. (1998), Ganias et al. (2004), Smith and Walker (2004) and Adebiyi et al. (2011).

Data analysis

All statistical analyses were carried out using SPSS software, version 17.0. Results are presented as mean±standard deviation.

Table. 1. Mean±SD body length, body weight, GSI, HSI, Genital papillae, MOS[1] and HOS[2] observed from May 2010 to December 2010 in female *P. hypophthalmus.*

Month	No. of fish sampled	Body weight (g)	Body length (cm)	GSI (%)	HSI (%)	Genital papilla	MOS[1]	HOS[2]
1(May)	16	284.00±24.02	33.17±0.97	0.29±0.09	1.51±0.15	Very tiny and silver	Immature	EPO
2(June)	11	323.72±31.62	34.12±1.13	0.26±0.07	1.59±0.13	Very tiny and silver	Immature	LPO
3(July)	13	393.00±39.75	35.72±1.14	0.28±0.04	1.56±0.09	Tiny and little pink	Immature	YV
4(Aug)	12	483.70±53.13	37.75±1.00	0.41±0.17	1.62±0.09	Tiny and pink	Maturing	YV
5(Sept)	10	666.00±53.80	40.67±0.93	0.44±0.14	1.74±0.05	Medium and pink	Maturing	PYG
6(Oct)	12	772.45±67.15	43.37±2.38	0.60±0.14	1.82±0.24	Medium and pink	Maturing	SYG/TYG
7(Nov)	14	989.70±59.98	46.99±1.04	0.77±0.14	2.04±0.14	Large and reddish	Mature	MN/HY
8(Dec)	13	1331.44±141.49	51.38±1.91	0.65±0.22	1.83±0.20	Large and reddish	Mature	MN/HY

MOS[1]: Macroscopic ovary stages. HOS[2]: Histological ovary stages. EPO: Early primary oocyte. LPO: Late primary oocyte. YV: Yolk vesicle. PYG: Primary yolk globule. SYG: Secondary yolk globule. TYG: Tertiary yolk globule. MN: Migratory nucleus. HY: Hydrated oocyte.

Results

Ovarian Morphology and GSI

Table 1 provides an overview of body weight, body length, GSI, HSI, genital papilla and ovarian morphological stage information recorded over the 8-month study period. During the immature stage, pink ovaries were seen as tiny, thin and transparent, paired organs close to the air bladder. The maturing development phase was identified when the ovaries appeared firm and elongated with a light pink colour and faint blood irrigation. Small oocytes were visible to the naked eye during this stage. The mature phase was characterised by an overall fresh ripe and transparent appearance of the ovary. The colour of the ovaries varied from reddish-pink to orange and the external wall was highly vascularised.

The GSI and HSI increased gradually from August to October and peaked in November. The lowest GSIs (%) were seen in the period May-July, the early part of the study, and the highest GSI was recorded in November. The lowest HSIs (%) were observed in May-July and the highest value (2.04±0.14) was recorded in November (Table 1).

Ovary histology and timing of maturity

Oocyte development in *P. hypophthalmus* was divided into eight stages as follows: early primary oocyte, late primary oocyte, yolk vesicle oocyte, primary yolk globule, secondary yolk globule, tertiary yolk globule, migratory nucleus and hydrated oocyte. Table 2 provides a description of the ovarian oocyte development stages based on the histological analysis of the sampled ovaries (Fig.1). The progress of oocyte development in the ovary of *P. hypophthalmus* over the 8-month study period is presented in Fig.2. Early primary oocyte and late primary oocytes were observed in all ovaries throughout the study but were not the dominant stages from month 4 onwards (Fig.2). In addition, the presence of oocytes in more than two developmental stages in a single ovary was also observed throughout the study period.

Table. 2. Description of oocyte developmental stages and timing of maturity progress.

Ovarian period	Developmental stages	Microscopic description
Previtellogenic	Early primary oocyte	Oogonia and perinucleolar oocytes present. Cytoplasm is dark blue colour. The oocyte was attained in large circular nucleus and peripheral nucleoli in May (Fig.1a).
	Late primary oocyte	Oogonia and perinucleolar oocytes are large in number. The oocytes appeared in circumnuclear ring stage and nucleus still with attached nucleoli in June (Fig.1b).
	Yolk vesicle oocyte	Small vesicles appear in the cytoplasm and then migrate to the periphery of the cytoplasm. The cortical vesicles increase in size and number to form several peripheral rows and zona radiata appeared in July to August (Fig.1c).
Vitellogenic	Primary yolk globule	A large number of oocytes with yolk granules and filled most of the cytoplasm. A central large irregular nucleus appeared in September (Fig.1d).
	Secondary yolk globule	Yolk granules enlarged in size and went to yolk globules oocyte with eccentric nucleus and advanced chorion in October (Fig.1e).
	Tertiary yolk globule	Yolk globules increase in number and filled two third of cytoplasm. The lipid droplets appeared empty in the mid to inner portion of the cytoplasm in October (Fig.1f).
Maturation	Migratory nucleus	Nucleus begins to leaves central position and migrates toward periphery in November to December. Yolk globules fill more than two third of cytoplasm. Oocyte size nearly remains stable (Fig.1g).
	Hydration	Yolk globules filled entire of cytoplasm. Nucleus split up and the numerous vacuoles observed within the cytoplasm. The yolk globules started to coalesce to form yolk plates in November to December (Fig.1h).

Confirmation of sexual maturity at sizes > 46.99 cm TL

The body lengths and weights of the collected fish were between 33.17 ± 0.97 to 51.38 ±1.91 cm and 284.00±24.02 to 1331.44±141.49 g respectively (Table 1). Immature ovaries with perinucleolar oocytes were rare at < 35.75 cm TL, while maturing stage with perinucleolar and previtellogenic oocytes were observed in females from 37.75 to 40.62 cm TL. The mature stage with primary, secondary and tertiary yolk globules oocytes in their early phase were found in females ≤ 43.37±2.38 cm TL, while mature ovaries with fully developed migratory nucleus and hydrated oocytes were visible in females ≥ 46.99±1.04 cm TL.

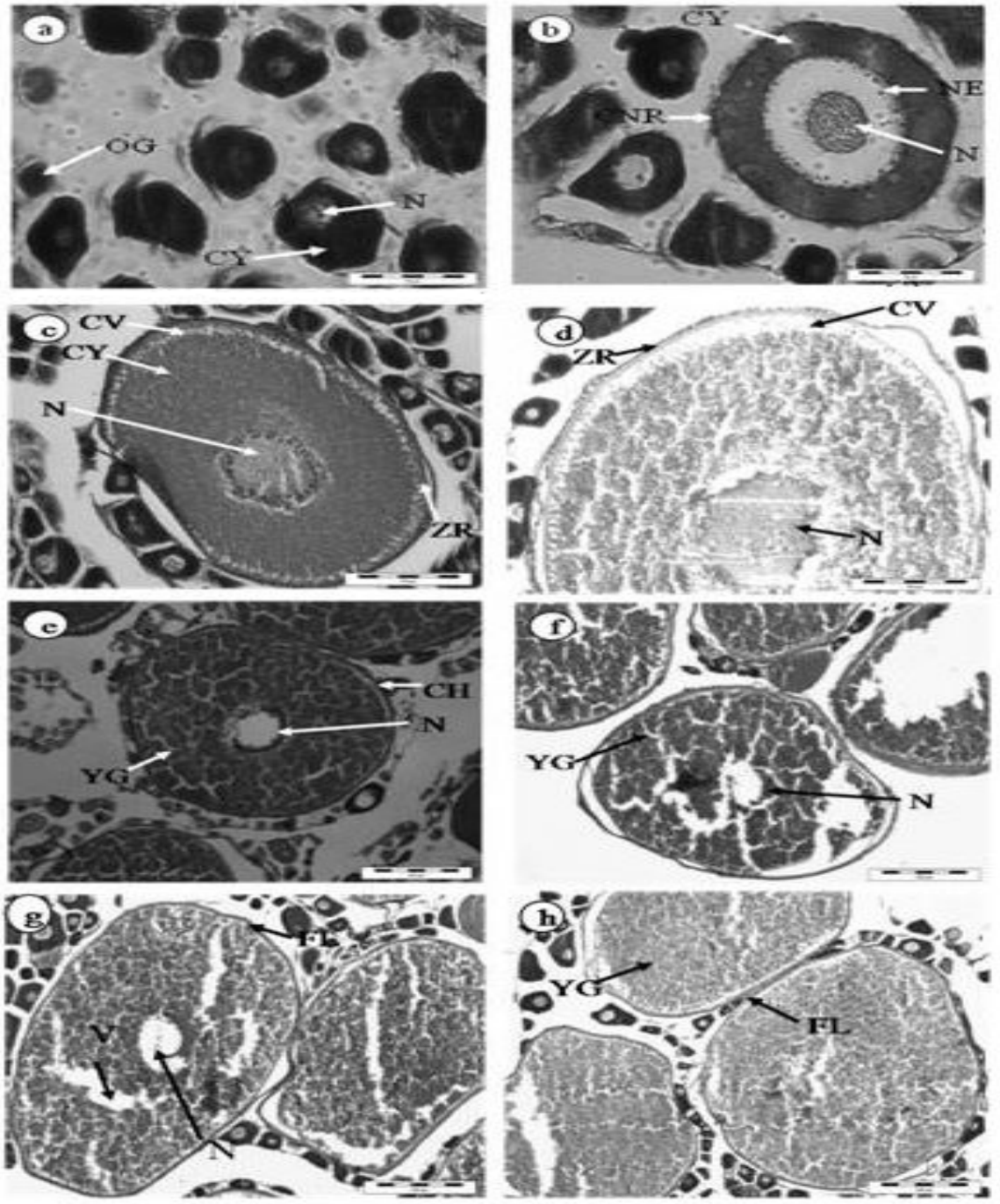

Fig.1. Ovary histological sections of different stages of *P. hypophthalmus* oogenesis. Previtellogenic growth phase (immature): **(**a) Early primary oocyte. (b) Late primary oocyte (c) Yolk vesicle oocyte. Vitellogenic growth phase (maturing): (d) Primary yolk globule oocyte. (e) Secondary yolk globule oocyte. (f) Tertiary yolk globule oocyte. Maturation growth phage (mature): (g) Migratory nucleus oocytes. (h) Hydrated oocyte.

N: Nucleus, OG: Oogonium, CY: Cytoplasm, NE: Nucleoli. CNR: Circumnuclear ring, CV: Corticol vesicle, ZR: Zona radiata, CH: Chorion, YG: Yolk globule, V: Vacuole, FL: Follicular layer.

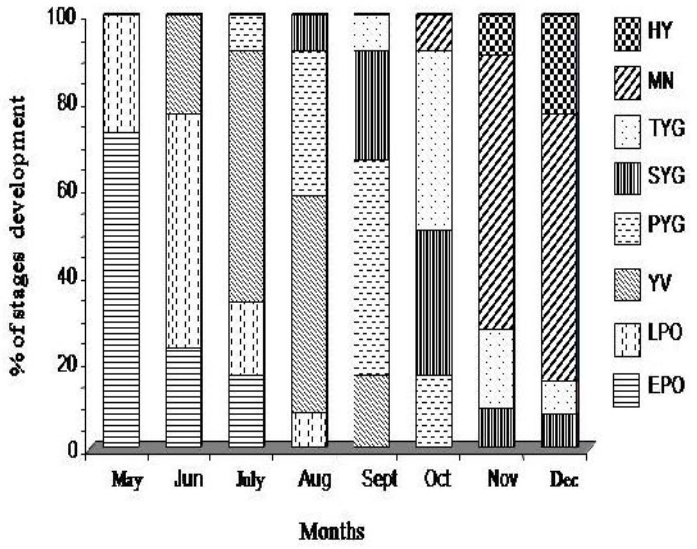

Fig. 2. Monthly progression of ovarian oocyte stages development in *P. hypophthalmus*. EPO: Early primary oocyte. LPO: Late primary oocyte. YV: Yolk vesicle. PYG: Primary yolk globule. SYG: Secondary yolk globule. TYG: Tertiary yolk globule. MN: Migratory nucleus. HY: Hydrated oocyte.

Discussion

Fish ovarian development and timing of maturation are important issues in aquaculture and may limit reproductive success. This study provides information on the ovarian development and sexual maturity of female *P. hypophthalmus* juveniles raised in captivity and monitored monthly to record oocyte development through morphological changes and histological analysis of ovary.

The three morphological stages, immature, maturing and matured, of the ovary of *P. hypophthalmus* observed in this study were similar to most teleost fish (Coward and Bromage 1998; Koc et al. 2008; Chelemal et al. 2009; Reidel et al. 2010; Adebiyi et al. 2011). Since ovary size in fishes increases with stage of development and with fish size and age, ovarian morphology and GSI provide useful tools to indicate oocyte growth and maturation (West 1990). In this study, GSI increased significantly with each morphological stage until the 7th month of culture period coinciding with the final stages of oocyte maturity and did not change significantly thereafter. It can therefore be inferred that spawning is likely to occur during this month. The highest GSI value (0.77%) recorded in November (7th month of the study, when fish were about 18 months old) was lower than the GSI of over 10% reported by Manosroi et al. (2004). Although the sizes of fish in these two studies were similar, the differences in GSI values could be due to factors such as the hormonal treatment which stimulates oocyte development (Peter and Yu 1997; Morehead et al. 1998; Mugnier et al. 2000; Manosroi et al. 2004), feeding and diet (Cek and Yilmaz 2009; Reidel et al. 2010) and rearing conditions (West 1990).

In this study, HSI values did not decrease during ovary development, indicating that the stored energy in liver was not utilised during this period and this is likely due to adequate provision of food to the fish held in captivity (Cek and Yilmaz 2009; Reidel et al. 2010) . Providing enough food to *P. hypophthalmus* during the study period might have played an important role in developing the ovary and liver. Similar findings have been reported at first maturity of female sharptooth catfish, *Clarias gariepinus* (Burchell, 1822) (Cek and Yilmaz 2009).

Knowledge of the ovarian developmental stages of fish is important in understanding fish reproductive performance for the purpose of developing a breeding programme. Generally, the stages of ovarian oocyte development in majority of fishes can be divided into five to eight stages (West 1990; Unal et al. 1999; Tomkiewicz et al. 2003; Ganias et al. 2004; Smith and Walker 2004; Cek and Yilmaz 2009; Reidel et al. 2010; Pham et al. 2011). Based on histological staging, oocyte development (oogenesis) of *P. hypophthalmus* in this study can be classified into eight stages (Table 2). The characteristics of the oocytes in the different stages were found to be similar to *Tilapia zillii* (Gervais, 1848), (Coward and Bromage 1998), Baltic cod, *Gadus morhua callarias* (Linnaeus, 1758), (Tomkiewicz et al. 2003), *Cyprinus carpio* Linnaeus, 1758, (Smith and Walker 2004), bagrid catfish, *Mystus nemurus* (Valenciennes, 1840), (Muchlisin et al. 2006), sharptooth catfish, *C. gariepinus* (Cek and Yilmaz 2009) and river catfish, *Hemibagrus nemurus* (Valenciennes, 1840), (Adebiyi et al. 2011). Although perinuclear oocytes were observed in all ovaries throughout the culture period, the EPO and LPO stages did not dominate the ovary from the 4th month onwards. This finding is in conformity with the reports of Babiker and Ibrahim (1979) in the cichlid, *Oreochromis niloticus* (Linnaeus, 1758), Bromage and Cumaranatunga (1988) in rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), and Adebiyi et al. (2011) in river catfish, *H. nemurus*, suggesting that pools of oogonia are always available for the continuous development of oocytes. Thus based on histological examination of the oocytes which showed the presence of up to four stages of oocyte development at various sampling times, it can be concluded that the mode of ovarian development of *P. hypophthalmus* is asynchronous. In aquaculture, asynchronous oocyte development has practical importance in establishing a breeding programme as it indicates a consistent supply of eggs to the hatchery. Developmental events observed in the oocytes of striped catfish, *P. hypophthalmus* are similar to those described for other species such as common carp (Smith and Walker 2004), *Xiphias gladius* Linnaeus, 1758 (Arocha 2002) and *T. zillii* (Coward and Bromage 1998).

In this study, minimal lengths and weights of females at first sexual maturity were ≥ 46.99±1.04 cm and ≥ 989.70±59.98 g respectively and these were observed between the 7th to 8th month of the experimental period and equivalent to an age of 1½ years since the fish were 6 months old at the start of the study. Sexual maturity in young *P. hypophthalmus* weighing approximately 1 kg has not been reported previously and differs from the current literature of 3-4 years or between 2-5 kg (Legendre et al. 2000) for *P. hypophthalmus* in the wild.

Although food may be readily available in the wild, the energy expended in search for food, and on other activities, instead of for reproductive development, may be the reason for the extended time taken for fish to mature in the wild. Time taken to achieve sexual maturity also tends to differ with culture conditions. In case of the common carp (*C. carpio*), Adamek et al. (1991) reported that sexual maturity occurred at 3-6 months or 90-140 mm standard lengths under controlled conditions, while Bishai et al. (1974) found that the time and size were between 3-5 years with a 355-430 mm TL for farmed fish in Egypt.

Conclusion

The macroscopic and histological analysis of the ovaries along with the examination of oocyte development showed that *P. hypophthalmus* can achieve oocyte maturity at an age of about 18 months in captivity provided sufficient food is available. The observations from this study with regard to ovarian oocyte development in *P. hypophthalmus* as well as its reproductive biology will contribute towards developing strategies for broodstock management in hatcheries.

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