The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Published under auspices of

The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)

&

University of Hawai'i at Mānoa

&

AquacultureHub

http://www.aquaculturehub.org

ISSN 0792 - 156X

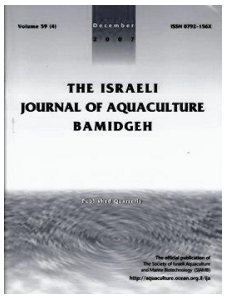
PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH - Kibbutz Ein Hamifratz, Mobile Post 25210, ISRAEL

Phone: + 972 52 3965809

http://siamb.org.il

The Israeli Journal of Aquaculture - Bamidgeh, IJA_68.2016.1326, 9 pages



Effects of Time after Hormonal Stimulation on Milt Properties in Waigieu Seaperch *Psammoperca waigiensis*

Minh Hoang Le*, Paul B Brown[1]

Institute of Aquaculture, Nha Trang University, Nha Trang city, Vietnam
[1]Department of Forestry and Natural Resources, Purdue University, 715 West State Street, West Lafayette, IN 47907-2061, USA

Keywords: Waigieu seaperch; hormonal stimulation; milt properties; LHRHa; HCG; DOM.

Abstract

The objective of the present study was to assess the effects of time after stimulation with domperidone (DOM), luteinizing hormone releasing hormone analog (LHRHa), and human chorionic gonadotropin (HCG), on features of spermiation including milt physical properties, sperm motility parameters, and sperm morphology, in Waigieu seaperch *Psammoperca waigiensis*. Male broodfish were injected with either 0.9% saline solution (control), or a single dose of DOM [20 mg/kg body weight (BW)], LHRHa (20, 50, or 80 µg/kg BW), or HCG (500, 1000, or 1500 IU/kg BW). Milt samples were collected before hormone induction (0 h) to assess original milt condition, and at 24 h, 48 h, and 72 h post injection (p.i.). Results showed that treatment with saline solution, DOM, or LHRHa did not significantly alter milt physical properties, sperm motility parameters, or sperm morphology. Treatment with HCG at a dose of 1000 IU/kg BW significantly increased milt volume, total sperm production, and sperm motility parameters, but decreased spermatocrit and sperm concentration at 48 h p.i. These parameters were significantly reduced at 72 h p.i. In conclusion, our results suggest that Waigieu seaperch milt should be collected 48 h after hormonal stimulation with a single dose of HCG (1000 IU/kg BW) to ensure high properties of milt such as milt volume, total sperm production, and sperm motility parameters.

* Corresponding author. Tel.: +84-905-465-811; Fax; +84-583-831-147; Email: mhle.vn@gmail.com or hoanglm@ntu.edu.vn

Introduction

Milt properties in fish can be affected by many factors such as nutrition, environmental factors (temperature, photoperiod, salinity), spawning season, stress, genetic materials, or hormonal stimulation (Bobe and Labbé 2010). Hormonal stimulation for artificial propagation in fish is frequently used as it may induce or enhance ovulation in females and spermiation in males. Several hormones are available and have been commonly studied in many fish species (Mañanós *et al.* 2009). Moreover, spermiation and ovulation in many marine fish species can be improved or increased following exogenous hormone stimulation, mainly human chorionic gonadotropin (HCG), luteinizing hormone releasing hormone analog (LHRHa), and domperidone (DOM) (Elakkanai *et al.* 2015). The results of these studies showed that proper hormonal stimulation in female fish is species-specific, but can be used for induction of male sperimation for all species.

Waigieu seaperch, *Psammoperca waigiensis* (Cuvier and Valenciennes, 1828), is a marine fish species in the order of Perciformes, family Latidae (or Centropomidae), which inhabit rocky or coral reefs, frequently in weedy areas, usually in holes and crevices by day, and distributed throughout tropical coastal waters in Asia Pacific and Australia (Pham *et al.* 2012). Because of its marketplace acceptance, economic value, and ability to exist in wide variations of environment (Pham *et al.* 2010a; Pham *et al.* 2012), it is a potential species for aquaculture. While seaperch can spawn in captivity without hormone stimulation, success of these spawns has been limited (Nguyen *et al.* 2003). The sexual maturation of Waigieu seaperch females occurs between April and October and in males between March and November (Pham *et al.* 2012). The effect of different exogenous hormones [thyroxin (T4), LHRHa, carp pitutary extract (CPE), HCG or DOM] on plasma testosterone levels, plasma concentration of 17β-estradiol, plasma 11-ketotestosterone levels and plasma progesterone levels in the female of this species have been reported (Pham *et al.,* 2010b). No information is available on the effects of exogenous hormones used for the enhancement of spermiation in captive broodfish, to ensure sufficient milt production for a long spawning season. The objective of the present study was to assess the effects of time after stimulation with DOM, LHRHa and HCG on spermiation in Waigieu seaperch *Psammoperca waigiensis,* and assess milt physical properties, sperm motility parameters, and sperm morphology, over a period of 72h post injection (p.i.).

Materials and methods

Fish and milt collection. Mature Waigieu seaperch of both sexes were captured in Nha Trang Bay, Vietnam between February and November 2012. Fish of both sexes were transported in oxygenated tanks to the floating net cage farm at Vung Ngan Bay, Nha Trang city, Vietnam. Fish were then kept in 4×4×3m floating net cages. The floating net cages were covered with nets to prevent escape. Broodfish were fed twice daily with trash fish such as squid and anchovy supplemented with vitamin E at 5% of body weight (BW) until the day before experiment.

Male broodstock sizes are shown in Table 1. Fish were anesthetized by immersion in 200 ppm ethylene glycol monophenylether in sea water before milt collection. Milt was collected into 1.5 ml eppendorf tubes by applying gentle pressure to the abdomen in an anterior to posterior direction. During milt collection, care was taken to avoid contamination of milt with urine, feces, blood, and mucous. Collected milt was kept on ice until analysis.

Table 1. *Body weight (BW, g) and total lengh (TL, cm) of Waigieu seaperch Psamoperca waigiensis used for experiments*

	0.9% saline (n=4)	*DOM (20 mg/kg BW) (n=4)*	*LHRHa (µg/kg BW)*			*HCG (IU/kg BW)*		
			20 (n=4)	*50 (n=4)*	*80 (n=4)*	*500 (n=4)*	*1000 (n=4)*	*1500 (n=4)*
BW	562.5±137.7	587.5±175.0	550.0±129.1	587.5±103.1	575.0±86.6	525.0±150.0	537.5±137.7	562.5±149.3
TL	26.8±1.4	26.9±1.7	26.8±1.4	27.0±1.1	27.0±1.1	26.5±1.6	26.5±1.4	26.6±1.4

DOM: domperidone; LHRHa: luteinizing hormone releasing hormone analog; HCG: and human chorionic gonadotropin

Experimental design. All experiments were performed between March and May 2015 at the floating net cages site. At the start of each trial (0h), thirty two males were randomly selected for milt collection as described above. Twenty-eight males were randomly injected with a single dose of either 20 mg/kg body weight (BW) of DOM or 20, 50, or 80 µg/kg BW of LHRHa (Lam Hua Dragon Co. Ltd., Hong Kong) or 500, 1000, or

1500 IU/kg BW of HCG (Ningbo Renjian Pharmaceutical Co., Ltd, China). Four other males were injected once with the 0.9% saline solution and served as control. Hormone doses were given on an incremental basis within ranges shown to be effective in tropical cultured marine fish species (Mañanós *et al.* 2009). Following injection, each male was held in a separate net cage (1x1x3m) and anesthetized after 24, 48, and 72 h. Milt was collected as described above. Milt properties such as physical properties, sperm motility parameters, and sperm morphology, were determined as described by Le *et al.* (2011a) and Le *et al.* (2014).

Milt properties analysis. Physical properties of milt included milt volume (ml per fish), sperm concentration in milt ($x10^9$ per ml), spermatocrit (%), and total sperm production ($x10^9$ per fish). Milt volume (MV) was determined by direct measurement from eppendorf tubes to the nearest 0.05 ml. Sperm concentration in milt (SCM) was determined using a hemocytometer counting chamber (Marienfeld, Germany) under a microscope (Olympus BX41TF, Tokyo, Japan) (X400 magnification). Spermatocrit (SM) was estimated using a Hawksley micro-hematocrit reader (Sons Ltd., England). Total sperm production (TSP) was calculated from MV and SCM data. Sperm motility parameters included percentages of motile sperm (MOT, %), velocity of average in path (VAP, µm/s), and duration of sperm motility (DSM, s). To measure these parameters, milt was diluted in artificial seawater (33 ppt) at the ratio 1:100. Then 1 µl of the diluted milt was placed on a glass slide (Teflon Printed Glass Slide; 21 wells; diameter of well, 4 mm; Funakoshi Co., Japan) with a cover slide, and observed immediately at 400× magnification under a microscope (Olympus BX41TF, Tokyo, Japan) connected to video camera (Nikon D5200, Japan). A sample was observed three times under the microscope and the time for each was estimated until sperm motility fell below 10%. To determine the MOT and VAP, recorded files were transferred to ImageJ software using computer aided sperm analysis (CASA) in the website http://rsb.info.nih.gov/ij/download.html. The DSM was calculated as the time taken for estimated proportion of sperm after activation until sperm motility fell below 10% (Le *et al.* 2011b; Lim and Le 2013).

Sperm morphology was determined using transmission electron microscopy (TEM) as described by Le *et al.* (2011a). The sperm samples were fixed for 24h in 2.5% glutaraldehyte medium in 0.1 M phosphate buffer medium (PBM, pH 7.2) at 4°C, rinsed in the same buffer, and plunged for 2h in 1.0% osmium tetroxide (OsO_4) in the PBM, then serially dehydrated with ethanol from 50% to 100% and embedded in Epon 8/2. Hardened blocks were sectioned at 60-70 nm-thickness and sections were mounted on copper grids. Samples were post-stained with 2% uranylacetate in 50% ethanol for 10 minutes and lead citrate medium for 5 minutes. Finally, the grids were examined and photographed using TEM (JEM-1230, Japan and JEOL 1010, JEOL Ltd. Japan).

Data analysis. Total length and body weight data are mean ± SD (standard deviation). All other data are mean ± SEM (standard error for mean). In order to determine the significance of differences between the kinds of hormone and dose for sperm motility parameters (MOT, VAP, DSM) and physical properties (MV, SCM, SM, TSP), one-way ANOVA and Duncan's post-hoc test ($p<0.05$) were applied. All statistical analyses were conducted using the SPSS software version 18.0.

Results

Milt physical properties. Treatment with LHRHa at doses of 20-80 µg/kg BW did not induce an increase in MV at any sampling time. Similarly, treatment with HCG at doses of 500 or 1500 IU/kg BW did not significantly improve the MV higher than saline solution (control) or DOM or LHRHa-treatments. Injection of HCG at 1000 IU/kg BW resulted in a significant increase in MV by 48h p.i. compared to the saline solution, DOM and LHRHa-treated fish (Fig. 1). SCM was significantly lower in the HCG 1000 UI/kg BW group at 48h p.i. when compared with other treatments (Fig. 2). SM was also lower in the HCG 1000 UI/kg BW group at 48h p.i. when compared with saline, DOM and LHRHa-treatments (Fig. 3). There was significant enhancement in TSP in the HCG 1000 IU/kg BW group at 48h p.i. over saline solution, DOM (20 mg/kg BW), HCG (500, 1500 IU/kg BW) and LHRHa (20, 50, 80 µg/kg BW) treatments (Fig. 4).

Sperm motility parameters. Injection of HCG at 1000 IU/kg BW resulted in a significant increase the sperm motility parameters MOT, VAP and DSM by 48h p.i. compared to other treatments (Fig. 5A, B, C, respectively).

Sperm morphology. The morphology of sperm was unaffected by hormone dose or type after the period of 72 h p.i. (Fig. 6 A-I, respectively).

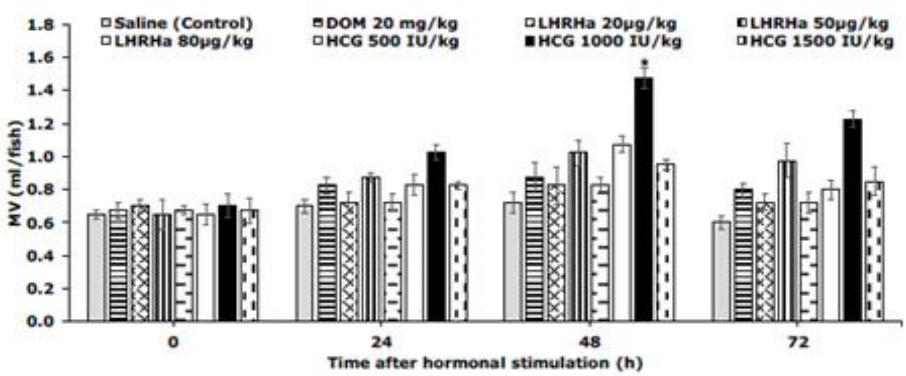


Figure 1. Milt volume (MV) produced by male Waigieu seaperch *Psammoperca waigiensis* injected with different doses of luteinizing hormone releasing hormone analog (LHRHa); human chorionic gonadotropin (HCG), domperidone (DOM), or saline solution (control). Values are presented as mean ± SEM. Values with different letters are significantly difference (ANOVA, P<0.05).

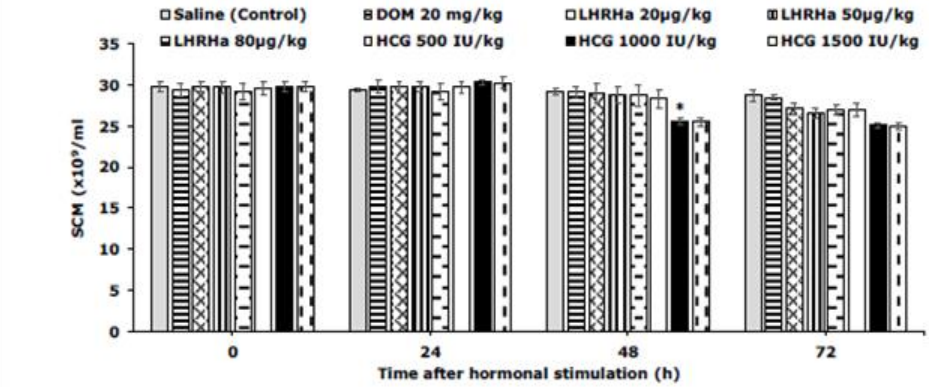


Figure 2. Sperm concentration in milt (SCM) from male Waigieu seaperch *Psammoperca waigiensis* injected with different doses of luteinizing hormone releasing hormone analog (LHRHa); human chorionic gonadotropin (HCG), domperidone (DOM), or saline solution (control). Values are presented as mean ± SEM. Values with different letters are significantly difference (ANOVA, P<0.05).

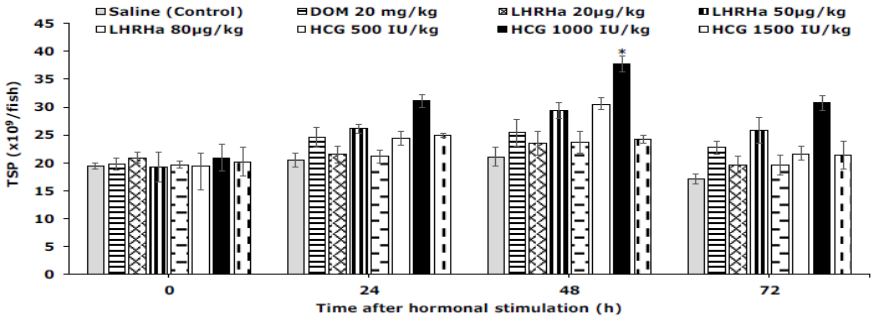


Figure 3. Total sperm production (TSP) from male Waigieu seaperch *Psammoperca waigiensis* injected with different doses of luteinizing hormone releasing hormone analog (LHRHa); human chorionic gonadotropin (HCG), domperidone (DOM), or saline solution (control). Values are presented as mean ± SEM. Values with different letters are significantly difference (ANOVA, P<0.05).

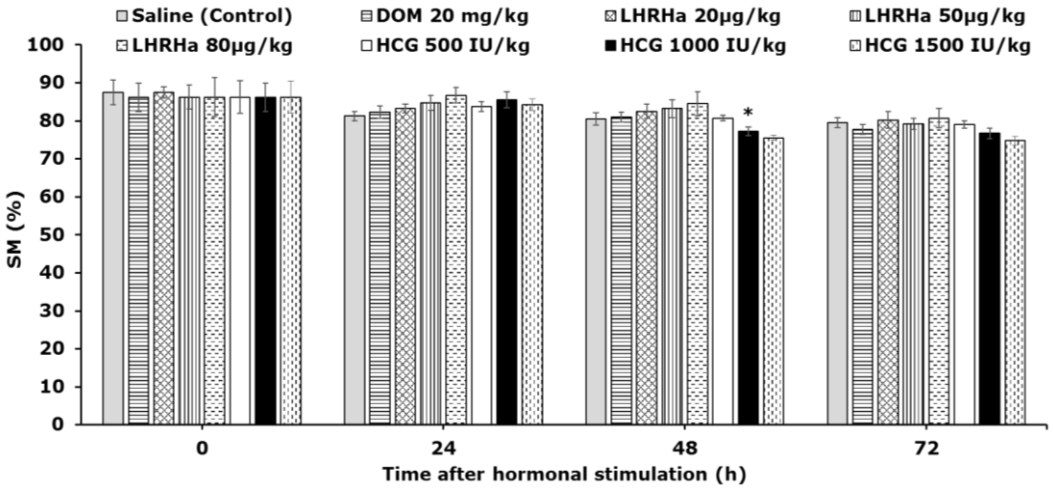


Figure 4. Spermatocrit (SM) from male Waigieu seaperch *Psammoperca waigiensis* injected with different doses of luteinizing hormone releasing hormone analog (LHRHa); human chorionic gonadotropin (HCG), domperidone (DOM), or saline solution (control). Values are presented as mean ± SEM. Value with star above is significantly difference (ANOVA, P<0.05).

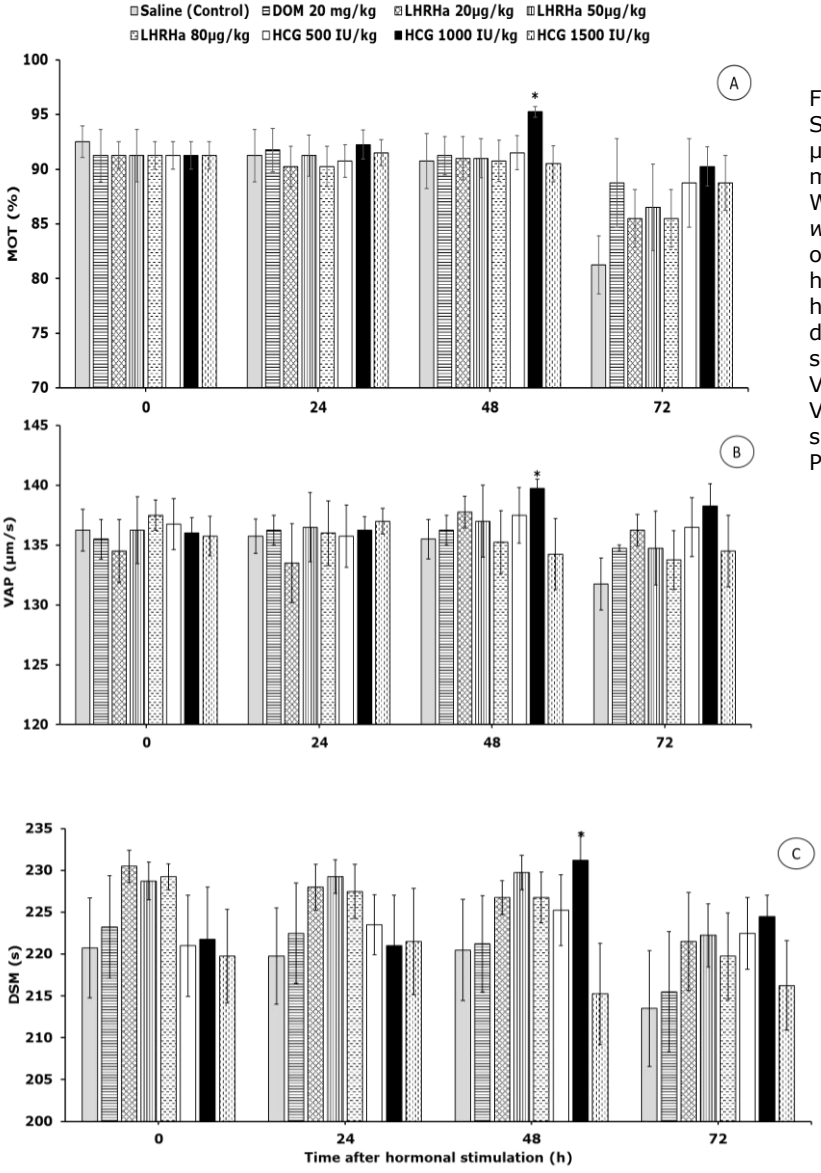


Figure 5. Motile sperm (A, MOT, %), Straight-linear velocity (B, VSL, µm/s) and duration of sperm motility (C, DSM, s) from male Waigieu seaperch, *Psammoperca waigiensis* injected with different doses of luteinizing hormone releasing hormone analog (LHRHa); human chorionic gonadotropin (HCG), domperidone (DOM), or saline solution (control). Values are presented as mean±SEM. Values with stars above are significantly different (ANOVA, P<0.05).

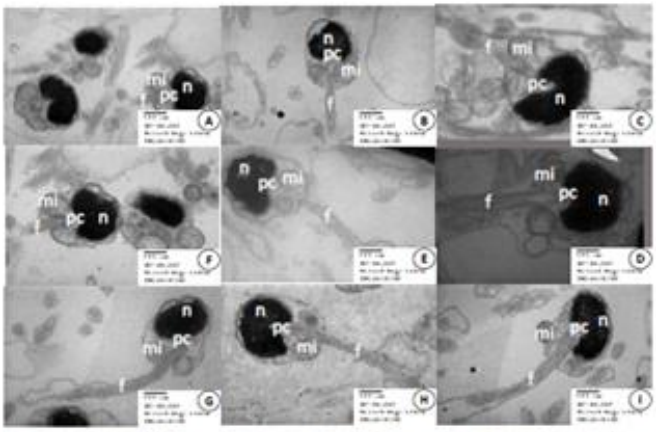


Figure 6. Effect of hormone injection on sperm morphology as tranmission electron microscropy (TEM) from Waigieu seaperch *Psammoperca waigiensis*. A. Before injection (0h); B. Saline solution injection after 72h; C. Luteinizing hormone releasing hormone analog (LHRHa) 20 ug/kg injection after 72h; D. LHRHa 50 ug/kg injection after 72h; E. LHRHa 80 ug/kg injection after 72h; F. HCG 500 IU/kg injection after 72h; G. human chorionic gonadotropin (HCG) 1000 IU/kg injection after 72h; H. HCG 1500 IU/kg injection after 72h. f: flagellum, mi: mitochondria, n: nucleus, pc: proximal centriole. Scale bar: 500 nm.

Discussion

The role of exogenous hormones on reproduction of teleosts has been studied and reviewed in order to ensure adequate milt production for artificial propagation (Elakkanai *et al.* 2015). In Vietnam, LHRHa, HCG, and DOM, are traditional agents for ovulation and spermiation induction in females and males of marine or freshwater fish species. Furthermore, gonadal development and spawning (ovulation in females and spermiation in males) in the marine or freshwater fish species is primarily controlled at three levels of the hypothalamus-pitutary-gonadal axis, because these organs produce substances influencing each other, leading to successful reproduction (Pham *et al.* 2010b). Consequently, various methods of hormonal stimulation are used in marine or freshwater fish species, the effectiveness being species-specific (Elakkanai *et al.* 2015).

MV and TSP in several fish species increased after hormonal stimulation, observed in paddlefish *Polyodon spatula* (Linhart *et al.* 2000), in tench *Tinca tinca* after inducing with 2 mg/kg BW carp pitutary extract (Caille *et al.* 2006), in striped bass *Morone saxatilis* (Mylonas *et al.* 1997b), in winter flounder *Pseudopleuronectes americanus* (Shangguan and Crim 1999), in white bass *Morone chrysops* (Mylonas *et al.* 1997a), in Atlantic salmon *Salmo salar* (King and Young 2001), in yellowtail bream *Acanthopagrus australis* (Black and Pankhurst 2009), in smelt *Osmerus eperlannus* after 48 h Ovaprim injection (Kowalski *et al.* 2012), in piabianha *Brycon insignis* after induction of PCE or GnRHa (Garcia *et al.* 2015), and in Asian seabass *Lates calcarifer* after 48 h LHRHa injection at a dose of 80 µg/kg BW (Hilomen-Garcia *et al.* 2002). However, the results of the present study showed that the MV and TSP attained after 1000 IU/kg BW HCG treatment increased, whereas injection of LHRHa (20, 50, 80 µg/kg BW) or DOM (20 mg/kg BW) or HCG (500, 1500 IU/kg BW) did not yield satisfactory results in stimulating Waigieu seaperch spermiation at 48h p.i. Similar results were reported for New Zealand snapper *Pagrus auratus* (Pankhurst 1994), and black porgy *Acanthopagrus schlegeli* (Yueh and Chang 1997). While one of the three hormones worked effectively for the MV and TSP of Waigieu seaperch, LHRHa 80 µg/kg BW (suitable hormone stimulation increase the MV and TSP for Asian seabass) resulted in low MV and TSP, suggesting the highly specific nature of hormones for various species. In this case, even though the Waigieu seaperch and Asian seabass are both in the same family, results after hormonal stimulation differ for increasing MV and TSP.

In contrast, SM and SCM in several fish species were reduced depending on the type, dose, time of hormone induction, spawning season, and fish species. Reduced SM and SCM were observed in winter flounder (Shangguan and Crim 1999), greenback flounder (Lim *et al.* 2004), Deccan mahseer (Basavaraj and Hedge 2005), and yellowtail bream (Black and Pankhurst 2009) after GnRHa stimulation. In our study, significantly lower SM and SCM compared to the others were shown after HCG 1000 IU/kg BW at 48h

p.i. This confirms that after hormonal stimulation in males, milt hydration takes place, but the rate of the process varies among species and depends on the hormone used (Bobe and Labbé 2010; Elakkanai *et al.* 2015). Significant differences in SCM or SM indicate that optimal hydration occurred following administration of HCG 1000 IU/kg BW at 48 h p.i in Waigeiu seaperch.

Sperm motility is one of the parameters used to estimate milt quality in marine and freshwater fish species (Browne *et al.* 2015; Felizardo *et al.* 2016). Hormonal stimulation in freshwater and marine species affected sperm motility (Elakkanai *et al.* 2015), but other studies showed that hormonal stimulation did not (Austriano *et al.* 2006). Our study showed that hormonal stimulation changed sperm motility as well as MOT, VAP and DSM. Sperm morphology of Waigieu seaperch did not change after hormonal stimulation at 72 h p.i. However, treatments in our study with HCG 1500 IU/kg BW and LHRHa 50 µg/kg BW in females of Waigieu seaperch at 48 h p.i. resulted in significantly increased spawning rate, but decreased fertility rates compared to thyroxin (T4) 0.5 mg/kg treatment via dietary or injection routes (Pham *et al.* 2010b). In the present study, these treatments applied to males of Waigieu seaperch at 48h p.i and resulted in low MV, TSP, MOT, VAP and DSM compared to HCG 1000 IU/kg BW treatment. Further experiments should be carried out with T4 treatment.

In conclusion, our results suggest that Waigieu seaperch milt should be collected 48h after hormonal stimulation with a single dose of HCG (1000 IU/kg BW) to ensure high milt properties.

Acknowledgements

This study was supported by project (106-NN.02-2013.69) at the National Foundation for Science and Technology Development (NAFOSTED). Thanks are extended to Dr. Ngo Van Manh and Ms. Vo Thi Thu Hien for technical assistance.

References

Austriano J.F., Mrco-Jimenez F., Perez L., Balasch S., Garzon Penaranda D.L., Vicente D.S., Viudes-deCastro J.S. and Jover M., 2006. Effect of HCG as spermiation inducer on European eel semen quality. *Theriogenology,* 66:1012-1020.

Basavaraj N. and Hedge S.N., 2005. Some characteristics and short-term preservation of spermatozoa of Deccan mahseer (Tor kludree). *Aquacult Res.,* 36:422-430.

Black B.J. and Pankhurst N.W., 2009. Effect of gonadotropin releasing-hormone analogue and human chorionic gonadotropin on milt characteristics and gonadal steroids in yellowfin bream, Acanthopagrus australis (Sparidae). *New Zealand J Marine and Freshwater Research,* 43:1069-1077.

Bobe J. and Labbé C., 2010. Egg and sperm quality in fish. *General and Comparative Endocrinology,* 165:535–548.

Browne R.K., Kaurova S.A., Uteshev V.K., Shishova N.V., McGinnity D., Figiel C.R., Mansour N., Agnew D., Wu M., Gakhova E.N. *et al.* , 2015. Sperm motility of externally fertilizing fish and amphibians. *Theriogenology,* 83:1-13.

Caille N., Rodina M., Kocour M., Flajshanc M. and Linhart O., 2006. Quantity, motility and fertility of tench (Tinca tinca) sperm in relation to LHRH analogue and carp pituitary treatment. *Aquacult. Int.,* 14:75-87.

Elakkanai P., Francis T., Ahilan B., Jawahar P., Padmavathy P., Jayakumar N. and Subburaj A., 2015. Role of GnRH, HCG and kisspeptin on reproduction of fishes. *Indian J Sci. Technol.,* 8:1-10.

Felizardo V.O.F., Mourad N.M.N., Melo C.C.V., Reis P.S., Murgas L.D.S. and Freitas R.T.F., 2016. Influential factors of quality of fish gametes for use of in vitro fertilization. *Journal of Fertilization: In Vitro - IVF-Worldwide, Reproductive Medicine, Genetics & Stem Cell Biology,* 4:166-169.

Garcia R.R.F., Vasconcelos A.C.N., Povh J.A., Ribeiro J.S.A., Eloy L.R. and Junior D.P.S., 2015. Seminal characteristics of piabanha before and after induction with different hormones. *Acta Scientiarum,* 37:399-403.

Hilomen-Garcia G.V., Baldevarona R.B. and Lacanilao F., 2002. Milt production of seabass *Lates calcerifer* administered an analogue of luteinizing hormone-releasing hormone and 17α-Methyltestosterone. *Isr. J. Aquacult.–Bamid., 54(4):173-182*.
King H.R. and Young G., 2001. Milt production by non spermiation male Atlantic salmon (*Salmo salar*) after injection of a commercial gonadotropin releasing hormone analoge preparation 17α-dihydroxy-4- pregnan-3-one alone or in combination. *Aquaculture,* 139:179-195.
Kowalski R.K., Hliwa P., Cejko B.I., Krol J., Stabinski R. and Ciereszko A., 2012. Quality and quantity of smelt *Osmerus eperlanus* sperm in relation to time after hormonal stimulation. *Reprod. Biol.,* 12:231-246.
Le M.H., Lim H.K., Min B.H., Lee J.U. and Chang Y.J., 2011a. Semen properties and spermatozoan structure of yellow croaker, *Larimichthys polyactis*. *Isr. J. Aquacult.-Bamid.,* 63:560:1-8.
Le M.H., Lim H.K., Min B.H., Park M.S., Son M.H., Lee J.U. and Chang Y.J., 2011b. Effects of varying dilutions, pH, temperature and cations on sperm motility in fish *Larimichthys polyactis*. *J Environ Biol.,* 32:271-276.
Le M.H., Nguyen T.H.N. and Pham P.L., 2014. Semen properties of waigieu seaperch *Psammoperca waigiensis*. *Isr. J. Aquacult.-Bamid.,* 66:7 pages.
Lim H.K. and Le M.H., 2013. Spermatozoa motile parameters in yellow croaker ***Larimichthys polyactis***: Effect of varying sugars, *Isr. J. Aquacult.-Bamid.,* 65:898:5 pages.
Lim H.K., Pankhurst N.W. and Fitzgibbon Q.P., 2004. Effects of slow release gonadotropin releasing hormone analog on milt characteristics and plasma levels of gonadal steroids in greenback flounder (*Rhombosolea tapirina*). *Aquaculture,* 240:505-516.
Linhart O., Mims S.D., Gomelsky B. and Hiott A.E., 2000. Spermiation of paddle fish (*Polyodon spathula*) stimulation with injection of LHRH analogue and carp pituitary extract. *Aquatic Living Resource,* 13:1-6.
Mañanós, E., Duncan, N. and Mylonas, C.C., 2009. Reproduction and control of ovulation, spermiation and spawning in cultured fish, in: Cabrita, E., Robles, V., Herráez, M.P. (Eds.), *Methods in Reproductive Aquaculture: Marine and Freshwater Species*. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, USA, pp. 3-80.
Mylonas C.C., Gissis A., Magnus Y. and Zohar Y., 1997a. Hormonal changes in male white bass (*Morone chrysops*) and evaluation of milt quality after treatment with a sustained-release GnRH delivery system. *Aquaculture,* 153:301-313.
Mylonas C.C., Scott A.P., Vermeirssen E.L.M. and Zohar Y., 1997b. Changes in plasma gonadotropin II and sex steroid hormones, and sperm production of striped bass after treatment with controlled-release gonadotropin-releasing hormone agonist-delivery systems. *J Biol Reprod.,* 57:669-675.
Nguyen T.N., Luc M.D. and Nguyen D.T., 2003. Studies on artificial seed breeding of waigieu seaperch *Psammoperca waigiensis* (Cuvier and Valenciennes, 1828). Components of scientific research and technological development between the University of Fisheries and SUMA Management, Ministry of Fisheries. *in Vietnamese*.
Pankhurst N.W., 1994. Effects of gonadotropin releasing hormone analogue, human chorionic gonadotropin and gonadal steroids on milt volume in the New Zealand snapper *Pagrus auratus* (Sparidae). *Aquaculture,* 125:185-197.
Pham Q.H., Kjørsvik E., Nguyen T.A., Nguyen D.M. and Arukwe A., 2010a. Reproductive cycle in female Waigieu seaperch (Psammoperca waigiensis) reared under different salinity levels and the effects of dopamine antagonist on steroid hormone levels. *J Exp Marine Biol Ecol.,* 383 137–145.
Pham Q.H., Nguyen T.A., Kjørsvik E., Nguyen D.M. and Arukwe A., 2012. Seasonal reproductive cycle in Waigieu seaperch (*Psammoperca waigiensis*). *Aquaculture Research,* 43:815-830.
Pham Q.H., Nguyen T.A., Nguyen D.M. and Arukwe A., 2010b. Sex steroid levels, oocyte maturation and spawning performance in Waigieu seaperch (*Psammoperca waigiensis*) exposed to Thyroxin, Human Chorionic Gonadotropin, Luteinizing Hormone Releasing Hormone and Carp Pituitary Extract. *Comparative and Biochemistry Physiology, Part A: Molecular & Integrative Physiology,* 155:223-230.

Shangguan B. and Crim L.W., 1999. Seasonal variation in sperm production and sperm quality in male winter flounder, *Pleuronectes americanus*: the effect of hypophysectomy, pituitary replacement therapy and GnRH-a treatment. *J Marine Biol.,* 137:19-27.

Yueh W.S. and Chang C.F., 1997. 17α, 20β, 21-trihydroxy-4-pregnen-3-one and 17α, 20β-dihydroxy-4-pregnen-3-one stimulated spermiation in protandrous black porgy, *Acanthopagrus schlegeli*. *Fish Physiol Biochem.* 17:187-193.