Combination effects human chorionic gonadotropin hormon and ovaprim distribution on the time latency, percentage of fertilization, hatching and survival of silver pompano *(Trachinotus blochii)* larve fish

IOP Conf. Series: Earth and Environmental Science **278** (2019) 012063 doi:10.1088/1755-1315/278/1/012063

Combination effects human chorionic gonadotropin hormon and ovaprim distribution on the time latency, percentage of fertilization, hatching and survival of silver pompano (*Trachinotus blochii*) larve fish

W K A Putra[1]* and A Mulah[1]

[1]Aquaculture Department, Faculty of Marine Science and Fisheries, Raja Ali Haji Maritime University, Riau Island

*E-mail: wiwin.bdp@umrah.ac.id

Abstract. Human Chorionic Gonadotropin (hCG) hormone has roles in inducing maturation, ovulation and fish spawning. Ovaprim is a reproductive hormone product that can stimulate the ovulation process and fish spawning. HCG hormone in marineculture is widely used for spawning fish with doses at around 1000 IU and 2 times of injections. This research was aimed to determine the effect of the combination of hCG and ovaprim hormone on spawning and figure the best treatment in inducing the spawning process of silver pompano. The method used four treatments: hCG x hCG, hCG x ovaprim, ovaprim x hCG, and ovaprim x ovaprim with three replicates of fish spawning (1 pair of parent fish). The best result in accelerating spawning latency time is ovaprim x ovaprim treatment and best performance is hCG x ovaprim treatment.

Keywords: hCG, ovaprim, spawing, silver pompano

1. Introduction

Spawning of silver pompano fish is influenced by several factors, namely hormone induction, environment and parent quality. Hormone induction in spawning silver pompano nodules is generally carried out twice, injecting the hormone hCG (day 1 before spawning) and hCG (day 2 before spawning). This is the main background of this research because the hormone hCG has more function on the maturation of the gonad until final maturation. Human Chorionic Gonadotropin (hCG) hormone is a hormone that has a dual function that can induce gonadal maturation and ovulation until spawning [1]. This is because the hormone hCG has a composition of liteunizing hormone (LH) which plays a role in the process of ovulation to spawning and follicle stimulating hormone (FSH) that plays a role in the gonadal maturation process. The influencing factors include the lack of preparedness of the parent for spawning, the low hatchability of the eggs, the survival of the larvae, and the lack of optimal parentization in spawning. One way to optimize the parent in spawning is by injection hormonal stimulation. Human Chorionic Gonadotropin (hCG) and ovaprim hormone. Both of these hormones are hormones that can induce optimal spawning of fish. The application of hCG hormone in spawning of silver pompano is done on the first injection (H1) and when it will be spawned (H-2) with a fairly high dose of 1,000 IU / inject / kg fish body weight. This proves inefficient use of the hormone hCG, where the hCG hormone is more appropriate to maximize gonadal maturation (egg and sperm cells).

Induction of the hormone hCG is not in accordance with the function for gonadal maturation. The hormone that has been commonly used for spawning fish is ovaprim but in spawning marine fish is

rarely used. Where ovaprim has a function as a fish spawning hormone. Hormones which are generally widely used to facilitate spawning are ovaprim. But the potential of hCG hormone which has a dual function and silver pompano is potentially developed to make a spawning hormone product and gonadal maturation in one product. The habit of using hCG and the problems above are the background of the study of the effect of hCG and ovaprim on the induction day 1 and 2 spawning of silver pompano fish.

2. Materials and Methods

2.1. Materials

The main ingredients used are the hormone hCG, ovaprim and the parent silver pompano (*Trachinotus blochii*) which is ready to spawn as many as 24 heads consisting of 12 males (size 1.5 kg) and 12 females (2.5 kg).

2.2. Procedure

This research was conducted using experimental methods, using Completely Randomized Design. The treatment that will be applied can be seen in table 1.

Table 1. Research design.

Reaplay	**Treatment**							
	T1		**T2**		**T3**		**T4**	
	Day1	**Day2**	**Day1**	**Day2**	**Day1**	**Day2**	**Day1**	**Day2**
R1	hCG	hCG	hCG	ovaprim	ovaprim	ovaprim	ovaprim	hCG
R2	hCG	hCG	hCG	ovaprim	ovaprim	ovaprim	ovaprim	hCG
R3	hCG	hCG	hCG	ovaprim	ovaprim	ovaprim	ovaprim	hCG

Preparation such as washing the tub and installing egg shelters, filling water and installing aeration stones. Selection of gonad maturity level with the aim to select a parent that is ready to be spawned, the selection is done by using a 4 mm catheter hose. Checking the quality of the parent by inserting a cannula hose into the urogenetic hole 2-3 cm then the hose is sucked and pulled slowly, and put into a glass bottle to be observed. If there is thick white liquid means is male and ready to be spawned, while for female parents the gonad is clear / yellowish in the form of granules and has been separated [2] Injections are carried out using the hormone hCG at a dose of 250 IU/kg and ovaprim 0.5 mL/kg in the right back, and a second injection is done to the pomfret tomorrow morning (23-24 hours) on the left back or vice versa. hCG is based on previous research, entitled Spawning Performance of Silver pompano with Comparison of Males and Females [3]. After the male and female mothers were injected they were collected in a spawning tank (2.5 m x 1 m x 1m). The parent was inserted with a sex ratio of 1:1. Then the spawning tank was closed using plastic to make the fish feel comfortable. Spawning usually occurs at night (between 18.00-24.00 WIB). Normally the parent will spawn after 12-41 hours from the last injection [4]. Parents who have spawned and/or not spawned are returned to their respective cages.

2.3. Research parameters

2.3.1. Spawning latency time. The length of time for spawning is calculated from the second injection until the egg is released in the spawning process. Checking is done once every 1 hour for 20 hours with an additional time of 4 hours. If the egg comes out more than 24 hours then the parent is considered not spawning and the latent time is 0 hours.

2.3.2. Fecundity. Spawning eggs will be collected in egg storage tanks. Eggs are harvested in the morning or 24 hours after spawning. and collected in an aquarium and the amount is calculated by

weighing. The eggs are taken a little then weighed up to 1 g then counted in 1 g, then all the eggs are weighed then multiplied by the number of eggs that have been counted in 1 g.

2.3.3. Fertilization rate. The eggs that are kept in the aquarium are observed and then counted by how many fertilized eggs. Calculation of percentage of fertilization rate based on formula [1]

$$FR = (\text{number of fertilized eggs}) / (\text{number of eggs released}) \times 100\% \quad (1)$$

Description: FR (fertile rate) degree of fertilization of eggs (%)

2.3.4. Hatching rate. The fertilized egg was observed until it hatched and then counted how many eggs hatched, then calculated using the formula. Calculation of percentage of hatching rate based on formula [1]

$$HR = (\text{number of eggs hatched}) / (\text{number of eggs in fruit}) \times 100\% \quad (2)$$

Description: HR (hatching rate) degree of hatching eggs (%)

2.3.5. Survival rate. Hatching eggs are maintained for 7 days to see the survival of the larvae. Calculation of the graduation percentage of the life of the larvae (survival rate) based on [5]

$$SR = Nt / No \times 100\% \quad (3)$$

Description: SR = (survival rate) level of survival of larvae (%)
Nt = Number of living larvae (tails)
No = Number of Larvae stocked (tail)

2.3.6. Data analysis. The research data of each parameter (latency time, percentage of spawning success, degree of fertilization rate, hatching rate, fecundity and survival rate of larvae) were analyzed descriptively. Results of each parameter are displayed in the form of graphs, tables or images.

3. Result and Discussion

Induction of the combination of human Chorionic Gonadotropin (hCG) and ovaprim hormones have an effect on the spawning of Silver pompano as can be seen in the parameters of latency time, percentage of successful spawning, fecundity, percentage of fertilization, percentage of hatching, and survival of larvae.

The results of the parameters of Silver pompano spawning latency time parameters, fecundity of treatments, fertilization percentage, hatching percentage, and survival rate can be seen in figure 1-5. Figure 1 shows that injecting ovaprim hormones on H-1 and ovaprim at H0 affects the latent time of spawning Siler pompano for 13.83 hours. These results were in accordance with the function of the ovaprim hormone, accelerating spawning and stimulating smooth muscle contraction to remove eggs and sperm. Ovaprim has LHRH and antidopamine hormone compositions, where LHRH plays a role in the process of ovulation and spawning by affecting the pituitary to produce LH hormone that will work until the fish spawning [6]. These results were compared with the treatment of injecting hCGxhCG, hCG x ovaprim, and ovaprim x hCG. silver pompano nirmal takes around 12-41 hours to spawn after the last injection [7].

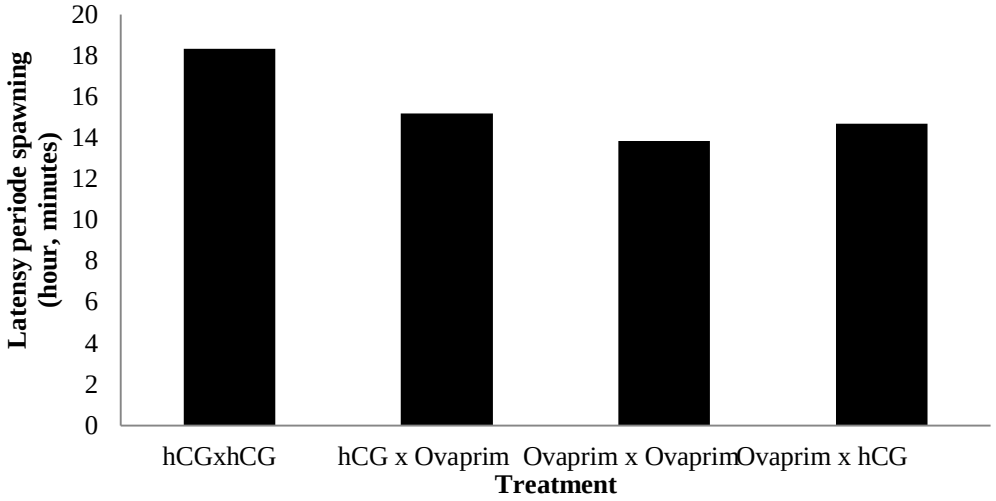

Figure 1. Spawning latency time.

.

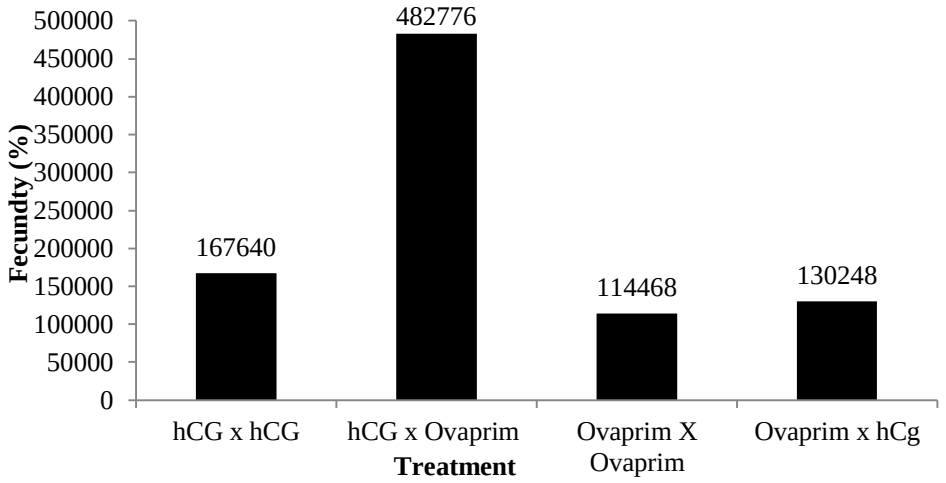

Figure 2. Fecundity of treatments.

Figure 2 shows that the best fecundity is the treatment of hCG x ovaprim with a total of 482,776 eggs. This is because the performance of the hCG hormone is a chorionic gonadotropin which has the properties of multiple biological activities, namely the effect of FSH and LH [8-10] the more dominant LH potential in hCG can improve the final gonad maturation and ovulation process in stellar fish while ovaprim performance able to give positive feedback to the hypothalamus and pituitary in promoting the release of Gonadotropin Releasing Hormone (GnRH). This release of GnRH will stimulate the pituitary in releasing LH. Increased LH in the fish body can increase the activity of 20β-hydroxysteroid dehydrogenase (20β-HSD) to produce 17α, 20β dihydroxyprogesterone so that oocyte maturation is followed by ovulation [6].

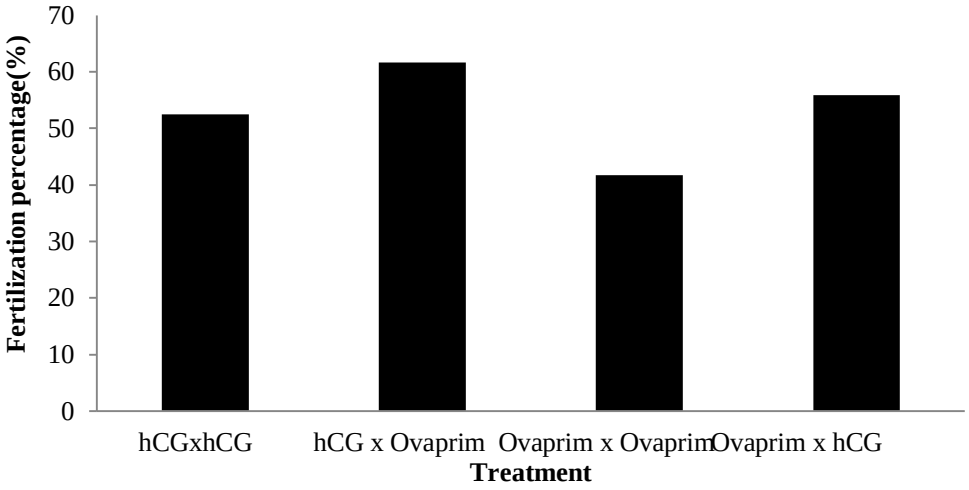

Figure 3. Fertilization percentage.

Figure 3 shows that the results of the best percentage of treatment was the 2nd treatment of hCG x ovaprim with a value of 61.7%. This value is far from good as it can usually reach 75% (Dikrurahman, 2010), but is very good compared to other treatments. This is because hCG contains LH and FSH which are gonadotropins I (GTH I), [11,12] that GTH I plays a role in increasing the secretion of Estradiol-17β which stimulates synthesis and secretion of vitelogenin. The use of hormones (sGnRHa + dopamine) not only encourages the mother to ovulate alone, but also affects the success of conception, hatching and the resulting larvae. Injecting the combination hormone hCG x ovaprim gives a very good value. This is due to the performance of the hCG hormone which helps maximize the maturation of the fish gonad itself, while the hormone that is given may also be too high in dosage. This is in accordance with the statement of [9, 13, 14]. Doses that are too high, turn out to have decreased results This is presumably because the mechanism of action of hormones will work normally (optimal) at a certain level, decrease or increase is expected to reduce the biological potential of hormones to the target.

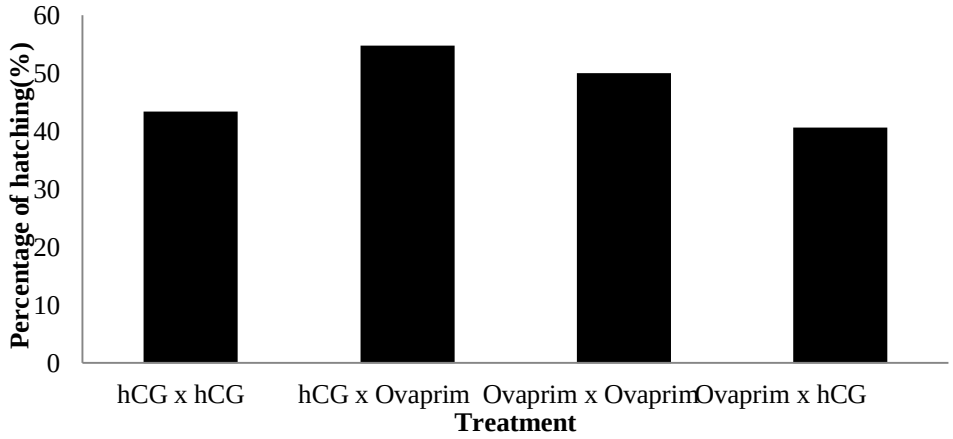

Figure 4. Hatching percentage.

Figure 4 shows that the best percentage of hatching eggs is treatment 2, namely hCG x ovaprim with a value of 54.8%. This is because the hormone hCG and ovaprim contain FSH. Caused by the content of Folicle Stimulating Hormone (FSH) increases so that the follicle develops and egg hatchability also increases [1,15,16] According to [17,18] fish eggs will usually develop normally if the hatchery conditions include oxygen, temperature, and pH. Some eggs often occur after a short period of development, namely the morula phase or before the closing of the blastoporosis. Oxygen deprivation is the reason for egg death. Temperature also kills eggs, usually during the embryonic development phase. At first the eggs look healthy and develop well. Over time, the eggs are white and dull. Fertilized

and unfertilized eggs are initially indistinguishable from fertilized eggs. Healthy eggs will develop into transparent or clear.

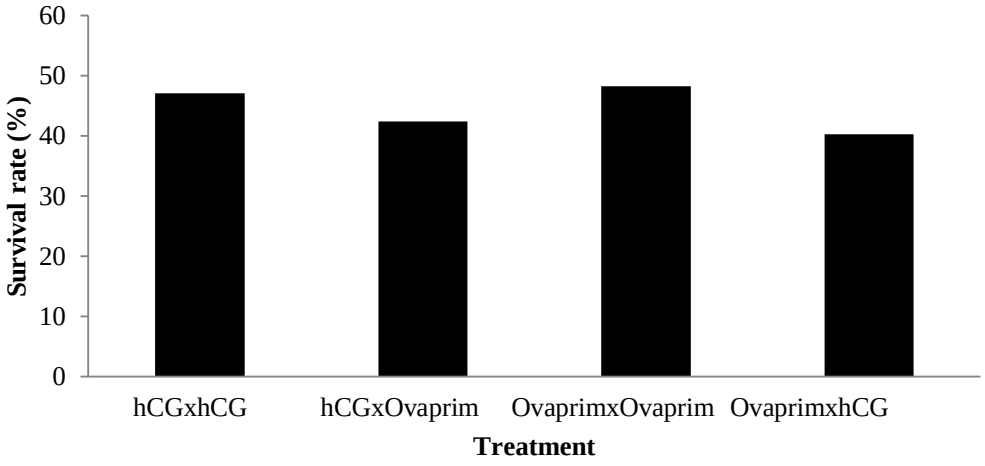

Figure 5. Survival rate.

Figure 5 shows that the survival of larvae is influenced by feed factors, one of which is the egg yolk itself and natural food. In this study the researchers observed these two things, the SR value obtained when larvae ate the third treatment egg yolk gave the highest value around 48.3% and when natural feed is given a change in value, the highest value is obtained in the second treatment, namely 28.4%. The results obtained are not much different from the previous research which stated that the SR produced during larval rearing ranged between 20-40% [5,19]. The survival of fish is influenced by internal and external factors [20]. Internal factors that influence are resistance to disease, feed and age. External factors that influence are stocking density, disease and water quality. Externally, stocking density is one of the most important factors because it is related to the movement of fish. When fish try to get fish feed, they will fight each other. If the unit area of the container used is narrow, the fish will be crammed together and can trigger the fish to stress out. When the fish condition is stressed, the fish not only lacks the response to the feed given and impacts on growth, but the fish will also be more susceptible to pathogens and even dead fish.

4. Conclusion

Effective treatment in accelerating the process of spawning of Silver pompano is the hormone hCG x ovaprim in this studyseen from the parameters of fecundity, percentage of breeding, percentage of hatching and percentage of larval survival. The quality of spawning results from the best treatment has latency time (15.17 hours), fecundity (482.777 items), fertilization rate (61.7%), hatching rate (54.8%), survival rate of larvae with egg yolk (42.4%) and survival of larvae with natural feed (28.4%).

References

[1] Weirich C R, Riley K L 2007 Volitional spawning of Florida Pompano, *Trachinotus carolinus*, induced via administration of gonadotropin releasing hormone analogue (GnRHa) *J. Appl. Aquac* 19(3) 47–60

[2] Putra F W K A, Raza'i T S, Zulfikar R T, Handrianto R 2018 Kajian pemijahan ikan Bawal Bintang (*Trachinotus blochii*) secara induksi hormona *Intek Akuakultur* **2** 54–62

[3] Kahkesh F B, Feshalami M. Y, Amiri F, Nickpey M, 2010 Effect of ovaprim, ovatide, HCG, LHRH-A2, LHRHA2+CPE and carp pituitary in Benni (Barbus sharpeyi) artificial breeding *Glob. Vet.* **5(4)** 209–214

[4] Jayakumar R, Nazar A K A, Tamilmani G 2014 Evaluation of growth and production performance of hatchery produced silver pompano *Trachinotus blochii* (Lacépède, 1801) fingerlings under brackishwater pond farming in India *Indian J. Fish* **61(3)** 58–62

[5] Chellapa K, Sakthivel M S, Tamilmani G, Kumar P R, Nazar A K B, Jayakumar R, Balamurugan, Ramkumar, Jothi P, Gopakumar G 2012 Survival and growth of juvenile silver pompano at different salinities in tropical condition *Indian J. Fish* **59(3)** 95–98
[6] Nagahama Y 2002 Endocrine regulation of gametogenesis in fish *Int. J. Dev. Biol* **38** 217–229
[7] Sharaf S M 2012 Effect of GnRHa , pimozide and Ovaprim on ovulation and plasma sex steroid hormones in African catfish Clarias gariepinus *THE.* **(77)8** 1709–1716
[8] Mulah A, Said T, Kusuma W, Putra A 2017 Efektivitas penggunaan Hormon human Chorionic Gonadotropin (hCG) dan ovaprim terhadap waktu latensi dan fekunditas dalam pemijahan ikan Bawal Bintang (*Trachinotus blochii) Intek Akuakultur* **1** 1–6
[9] Handrianto R, Said T, Kusuma W, Putra A 2017 Pengaruh hormon human Chorionic Gonadotropin (hCG) dan Pregnant Mare Serum Gonadotropin (PMSG) terhadap pematangan gonad ikan Bawal Bintang (*Trachinotus blochii*) *Intek Akuakultur* **1(2)** 16–22
[10] Mollah M F A, Tan E S P 1983 HCG-induced spawning of the Catfish, *Clarias macrocephalus* (Gunther) *Aquaculture* **(35)C** 239–247
[11] Selavaraj, Francis T , Venkatasamy M, Santhoshkumar S 2012 Effect of Hcg implants on change in testosterone and estradiol level in blood serum of Murrel , *Channa Striatus Tamilnadu J. Veterinary and Animal Science* **8(1953)** 290–298
[12] Devlin R H, Nagahama Y 2002 Sex determination and sex differentiation in fish : an overview of genetic, physiological, and environmental influences *Aquaculture* **208** 191–364
[13] Kim D, Suzuki Y, Aida K 2011 The control mechanism of gonadotropin-releasing hormone and dopamine on gonadotropin release from cultured pituitary cells of Rainbow Trout *Oncorhynchus mykiss* at different reproductive stage *Korean Society Fish Aqua Sci* **14(4)** 379–388
[14] Goo I B, Park I, Gil H W, Im J H 2015 Stimulation of spermiation by human chorionic gonadotropin and Carp Pituitary extract in Grass Puffer (*Takifugu niphobles) Korean Society Dev. Reprod.* **19(4)** 253–258
[15] lntaramongkol I C, Techakumphu S I M, Lohachit C, Tantasuparak I W, Ovarian responses and oocyte recovery inprepubertal Swamp Buffalo (*Bubalus bubalis*) Calves After Fsh. Or. Pmsg. Treatment *Theriogenology* 305–312
[16] Wu Y, He Z, Zhang L, Jiang H, Zhang W 2012 Ontogeny of immunoreactive Lh and fish cells in relation to early ovarian differentiation and development in Protogynous Hermaphroditic Ricefield Eel *Biology of reproduction* **86(3)** 1–9
[17] Usman B I, Amin S M N, Arshad A, Rahman M A 2013 Fecundity and egg size of grey-eel catfish *Plotosus Canius* (Hamilton, 1822) from the coastal waters of Kampong Telok, Negeri Sembilan Peninsular Malaysia *Asian J. Anim. Vet. Adv.* 8(2) 348–354
[18] Dimaggio M A, Broach J S, Ohs C L 2014 Evaluation of ovaprim and human chorionic gonadotropin doses on spawning induction and egg and larval quality of Pigfish, *Orthopristis chrysoptera Journal of The world Aquaculture Society* **45(3)** 243–257
[19] Patil D W, Singh H 2014 Effect of papain supplemented diet on growth and survival of post - larvae of *Macrobrachium rosenbergii International Journal of Fisheries and Aquatic Studies* **1(6)** 176–179
[20] Joseph T, Ii D 2017 Supplementing broodstock and larval diets for Florida Pompano [Thesis] Auburn University, Alabama USA