



RESEARCH NOTE

Induced spawning of siban fish *Cyclocheilichthys apogon* using Ovaprim [version 1; referees: 2 approved, 1 approved with reservations]

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v1 First published: 18 Oct 2017, 6:1855 (doi: [10.12688/f1000research.12885.1](https://doi.org/10.12688/f1000research.12885.1))
 Latest published: 18 Oct 2017, 6:1855 (doi: [10.12688/f1000research.12885.1](https://doi.org/10.12688/f1000research.12885.1))

Abstract

Background: The objective of the present study was to examine the effect of Ovaprim dosage on the latency period, relative number of ovulated eggs, fertilization, hatching, and survival rates of the siban fish, *Cyclocheilichthys apogon*.

Methods: Three dosages of Ovaprim were tested in this study, namely 0.3 ml kg⁻¹ of broodfish body weight, 0.5 ml kg⁻¹ body weight, and 0.7 ml kg⁻¹ body weight, plus control (without Ovaprim).


Results: The results showed that the best latency period, relative number of ovulated eggs, fertilization, hatching, and survival rates were obtained at a dosage of 0.7 ml kg⁻¹ body weight.

Conclusions: The best dosage of Ovaprim for siban fish from the dosages tested, was determined to be 0.7 ml kg⁻¹ body weight.

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Referee Status:   

	Invited Referees		
	1	2	3
version 1 published 18 Oct 2017	 report	 report	 report

- 1 **Javad Ghasemzadeh** , Chabahar Maritime University, Iran
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- 3 **Agung Damar Syakti**, Jenderal Soedirman University, Indonesia

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Author roles: **Nuraini N:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Writing – Original Draft Preparation; **Tanjung A:** Formal Analysis, Investigation, Methodology, Validation, Visualization;

Warningsih T: Data Curation, Formal Analysis, Investigation, Resources, Software; **Muchlisin ZA:** Resources, Software, Supervision, Validation, Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

How to cite this article: Nuraini N, Tanjung A, Warningsih T and Muchlisin ZA. **Induced spawning of siban fish *Cyclocheilichthys apogon* using Ovaprim [version 1; referees: 2 approved, 1 approved with reservations]** *F1000Research* 2017, **6**:1855 (doi: [10.12688/f1000research.12885.1](https://doi.org/10.12688/f1000research.12885.1))

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Grant information: This study was supported by Universitas Riau through thr PUPT scheme 2016, Grant number: 023/UN.5.1.3/PP/2016. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

First published: 18 Oct 2017, **6**:1855 (doi: [10.12688/f1000research.12885.1](https://doi.org/10.12688/f1000research.12885.1))

Introduction

Siban fish *Cyclocheilichthys apogon* is a commercial freshwater fish in Indonesia. This species is distributed in South East Asia regions such as Sumatra, Borneo, Malaysia, and Mekong, Thailand¹. Siban fish is one of the main targets of local fishermen in Riau Province, Indonesia, resulting in decreasing wild populations over the last decade (Personal communication with local fishermen of the Kampar River). Therefore, the cultivation of siban fish needs to develop in relation to meeting the market demand, without disturbing the wild population.

Nurhusniah² and Sari³ have studied the reproductive biology of the siban fish, but no other aspect has been studied yet. Nonetheless, the culturing of the siban fish has been initiated in Riau Province, Indonesia; the larvae were collected from the wild at low quality and quantity, with high seasonal dependence⁴, resulting in low production. Therefore, the breeding technology of siban fish is crucially needed to overcome these problems and to support the aquaculture business of the local people.

Induced spawning is one of the common methods to stimulate ovulation of the fish in the hatchery. With this method, hormones are playing an important role⁵. Several natural and artificial hormones have been used to induce breeding of fish, while Ovaprim is one of the most popular and effective solutions to stimulate the maturation of male and female broodfish. Ovaprim contains combinations of salmon gonadotropin-releasing hormone (sGnRH) and domperidone. These hormones have been applied successfully to induce spawning of seukuran fish *Osteochilus vittatus*⁶, selais *Ompok hypophthalmus*^{7,8}, common carp *Cyprinus carpio*⁹, mali-mali *Labiobarbus festivus*¹⁰, and lelan fish *Osteochilus pleurotaenia*¹¹.

Ovaprim has several advantages, for example, it is cheap, has many practical uses, and it is easy to find in the local market in Indonesia. However, Ovaprim has never been used to induce spawning of siban fish. Hence, the objective of the present study was to determine an effective dosage of Ovaprim for siban female broodstock.

Methods

Study timeline and site

The experiments were carried out within the ethical guidelines in animal research developed by NC3Rs. The study was conducted between April and June 2016, at the Fish Breeding Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia. A total of 12 male and 12 female broodfish of the siban fish *Cyclocheilichthys apogon* species were collected from the Kampar River, Riau Province. The broodfish ranged between 10 and 15 cm in total length, and 18.42 and 31.15g in total weight. The fish were acclimatized for 24 hours prior to inducing with Ovaprim.

Hormone administration

The experiment was carried out in compliance with the ethical guidelines provided by the Research Institution of Riau University (SOP/02/PL/LPPM/2016). Three dosages of Ovaprim

(Syndel, Canada) were tested in this study: for females, 0.3 ml kg⁻¹ body weight (BW), 0.5 ml kg⁻¹BW, and 0.7 ml kg⁻¹BW; for males, 0.15 ml kg⁻¹BW. The broodfish in control groups were injected with physiological solution (0.9% NaCl) at the dosage of 0.25 ml kg⁻¹BW.

Sperm and egg collection

The female broodfish were injected with their respective dosage two times; the first injection was at 8.00 PM with half of the tested dosage, and the second injection was conducted 6 hours after the first injection (at 2.00 AM), with the remainder of the tested dosage. The female broodfish was ovulated 6 hours after the second injection (at 8.00 AM). The eggs were collected by gentle finger pressure to the abdomen. The collected eggs were put in a plastic jar and kept in an ice box at 4°C⁶.

The males were injected with a single dose of 0.25 ml kg⁻¹ BW at 8.00 PM. The male siban fish was sacrificed with MS-222 (Merck), prepared by dissolving 4g of MS-222 in 5L tap water¹². The testes were removed and washed with physiological solution and then perforated and chopped with scissors. The semen was gently squeezed out and put in a tube, and kept in an icebox (4°C). The semen was mixed with physiological solution (0.9% NaCl) at dilution ratio 1: 100 (v/v).

Fertilization and incubation

A total of 1 ml eggs and 1 ml of diluted sperm were mixed homogenously in a plastic basin (Calista, Volume 500 mL) and with approximately 1 ml of fertilization solution (contains 4 g urea and 3 g NaCl L⁻¹) developed by The Laboratory of Fish Breeding, Faculty of Fisheries and Marine Sciences, Universitas Riau. The resulting mixture was left in contact for 5 minutes. The incubation basin was installed with 24-hour portable aerator and LED lamp.

A total of 100 eggs were taken randomly and then incubated in a plastic basin (Calista, volume: 2L), with three replicates at a water temperature of 25–27°C in a water heater. Successful fertilization was observed 8 hours after incubation. Unfertilized eggs were identified by their opacity; the unfertilized eggs were removed from the jar, while hatching rate was monitored at two-hour intervals¹³. The larvae were fed on *Tubifex* sp. *ad libitum* on day 5 after being hatched and reared in the same jar for 40 days.

Measured parameters and data analysis

The latency period, the relative number of ovulated eggs, egg diameter, fertilization rate, hatching rate, and survival rate of larvae on day 40 after hatching were measured. The latency period was determined by calculating the time between the second injection and ovulation. The relative number of ovulated eggs was calculated by dividing the total number of released eggs after Ovaprim injection by the total body weight of the female broodfish. Fertilization, hatching, and survival rates were calculated based on Muchlisin *et al*^{6,14} and Adami *et al*¹⁵. All data were subjected to One-Way Analysis of Variance (ANOVA), followed by the Duncan multiple-range test.

Results

The ANOVA test revealed that different Ovaprim dosages had a significant effect on latency period, relative number of ovulated eggs, fertilization, hatching, and survival rates ($p < 0.05$). The result showed that the latency period was faster at a dosage of 7 ml kg⁻¹BW; this value was significantly different from other dosages. A higher relative number of ovulated eggs and fertilization, hatching, and survival rates were also recorded at the Ovaprim dosage 7 ml kg⁻¹ BW; these values are significantly different from other dosages (Table 1). In general, the relative number of ovulated eggs, fertilization, hatching, and survival rates were increased with increasing Ovaprim dosage. However, there were no significant differences between the values seen at 3 ml kg⁻¹ BW and 5 ml kg⁻¹ BW dosage.

Dataset 1. Raw data collected for the latency period, relative numbers of ovulated eggs, fertilization, hatching and survival rates of the siban fish *Cyclocheilichthys apogon*

<http://dx.doi.org/10.5256/f1000research.12885.d180825>

Discussion

The results indicate a decreased latency period at increased Ovaprim dosage. On the contrary, the relative number of ovulated eggs, fertilization, hatching, and survival rates were increased at increased dosage. The best results for all parameters were recorded at the Ovaprim dosage of 7 ml kg⁻¹ body weight. Therefore, the higher dosage of Ovaprim (7 ml kg⁻¹ BW) was an effective dosage in inducing spawning of the siban fish compared to the lower dosage (3 ml kg⁻¹ and 5 ml kg⁻¹). This is probably because the combination of sGnRH and domperidon in Ovaprim solution at higher doses of 7 ml kg⁻¹ BW stimulated gonadotropin (GTH II or LH) secretion in the pituitary gland of the broodfish to a greater extent, and then induced gonad maturation and ovulation. The higher levels of gonadotropin will induce ovulation faster¹⁶. According to Ithisom⁹, the hormone works optimally at a certain dose; and changing the dose will reduce effectiveness. Therefore, determining the optimum dose is crucial.

The influence of sGnRH and domperidon at the higher dosage most likely inhibited secretion of dopamine and stimulated the pituitary gland to secrete GTH. The GTH in this case, GTH II, would stimulate the theca cells to secrete hormone 17-alpha hydroxyprogesterone, which would then be converted into maturation-inducing steroid by the enzyme 20-beta-dihydroxy steroids, stimulating follicles to burst as the oocytes hydrate^{17,18}.

The results showed that the high fertilization rate was obtained at doses of 0.7 ml kg⁻¹ BW; this dosage probably gave the optimum effect of sGnRH and domperidone to increase the quantity and quality of eggs. This is indicated by the higher number of ovulated eggs as recorded at this dosage. According to Nuraini *et al.*¹⁹ the optimum dosage of stimulating hormone results in better ovulation and improves egg quality for the selais fish, *Ompok hypophthalmus*. Moreover, Woynarovich and Horvath²⁰ stated that the fertilization rate is strongly influenced by the quality of eggs and spermatozoa. Prostaglandin plays an important role in inducing ovulation and significantly influences fertilization and hatching rates. This is because prostaglandin contains arachidonic acid derived from essential fatty acids that determine the egg quality²¹. In addition, the quality of eggs is influenced by several factors including the hormone levels²², nutrition²³, genetics²⁴ and the environment²⁵. Bromage *et al.*²⁶ stated that the quality of eggs is reflected in higher fertilization and hatching rates.

Besides being influenced by hormonal factors, the hatching rate is also influenced by the temperature of the incubation media, dissolved oxygen, pH, and light intensity²⁰. In general, the survival rate of siban larvae was categorized as being at a good level. According to Alikunhi *et al.*²⁷ there are three levels of survival rate for the larvae: a good level when the survival is higher than 50%, moderate when between 30 and 50%, and low when the survival is lower than 30%. However, the survival of the larvae in this study was lower compared to seurukan fish *Osteochilus vittatus* and river catfish *Mystus nigricap*, which were injected with Ovaprim 0.5 ml kg⁻¹ BW, resulting in a survival rate of between 80.66 and 90%^{6,28,29}. It is assumed that the siban fish requires higher dosages of Ovaprim to induce gonad maturation as recorded in this study.

Table 1. Latency period, fertilization, hatching, and survival rate of siban fish *Cyclocheilichthys apogon* based on Ovaprim dosages.

No	Parameter	Ovaprim dosage (ml kg ⁻¹ body weight)			
		0	3	5	7
1	Latency period (hour)	10.01±0.01 ^d	7.27±0.06 ^c	7.18±0.04 ^b	7.07±0.04 ^a
2	The relative number of ovulated eggs (eggs g ⁻¹ of BW)	5.5±0.71 ^a	10.67±6.03 ^b	11.00±0.00 ^b	19.00±11.53 ^c
3	Fertilization rate (%)	40.00±1.41 ^a	60.00±8.19 ^b	60.00±7.55 ^b	76.67±9.07 ^c
4	Hatching rate (%)	25.55±2.12 ^a	62.94±2.05 ^b	56.95±5.31 ^b	76.00±2.92 ^c
5	Survival rate on day 40 after hatched (%)	40.00±2.83 ^a	61.95±1.80 ^b	58.60±1.08 ^b	79.99±9.13 ^c

Conclusions

It is concluded that different doses of Ovaprim had a significant effect on the latency period, the relative number of ovulated eggs, fertilization, hatching, and survival rates of siban fish. The best Ovaprim dosage, from the ones tested, was 0.7 ml kg⁻¹ BW.

Data availability

Dataset 1: Raw data collected for the latency period, relative numbers of ovulated eggs, fertilization, hatching and survival rates of the siban fish *Cyclocheilichthys apogon*. DOI, [10.5256/f1000research.12885.d180825](https://doi.org/10.5256/f1000research.12885.d180825)³⁰.

Competing interests

No competing interests were disclosed.

Grant information

This study was supported by Universitas Riau through the PUPT scheme 2016, Grant number: 023/UN.5.1.3/PP/2016.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

The authors thank the rector of Universitas Riau for providing the research grant to support this study.

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Current Referee Status:



Version 1

Referee Report 14 November 2017

doi:10.5256/f1000research.13967.r27098



Agung Damar Syakti

Center for Maritime Biosciences Studies, Institute for Sciences and Community Service, Faculty of Fisheries and Marine Science, Jenderal Soedirman University, Purwokerto, Indonesia

This manuscript studied the effect of Ovaprim dosage on the latency period, relative number of ovulated eggs, fertilization, hatching, and survival rates of the siban fish, *Cyclocheilichthys apogon*. Scientifically, overall quality of the manuscript is interesting and good. This work provides insight into the best latency period, relative number of ovulated eggs, fertilization, hatching, and survival rates of the optimum dosage of Ovaprim. Thus, from my point of view, this paper can provide some important scientific data and values. I can recommend the acceptance and indexing of this manuscript in *F1000Research*. However, there is some specific comment and queries to be considered and it is necessary to pay close attention to proper sentence structure and grammar in some sentences of the manuscript.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

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Are sufficient details of methods and analysis provided to allow replication by others?

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If applicable, is the statistical analysis and its interpretation appropriate?

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Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 14 November 2017

doi:10.5256/f1000research.13967.r27096



Suparno Pranoto

Faculty of Fisheries and Marine Sciences, University of Bung Hatta, Padang, Indonesia

Title:

The title is good, brief and informative, you need to add the fish and the author name, also the year.

Abstract:

The conclusion still states the result of the research

Introduction:

In the introduction, give the information which is relevant with the **Induced spawning of siban fish *Cyclocheilichthys apogon* using Ovaprim.**

Suggestion: The introduction should have information of previous research about siban fish reproduction with specifically, the latest reference and the novelty about the research.

Methods:

Please add the research design. Method of research must be added with the clear reference.

Results:

Please describe the table 1 and emphasize the findings. Findings on the latency period, relative number of ovulated eggs, fertilization, hatching, and survival rates of the siban fish.

Discussion:

Discussion must be based on the emphasis on the findings, and relation of the findings with the basic concepts. Is there any contradiction and suitability with other peoples research which is related with using ovaprim to siban fish?

Conclusion:

Please write the conclusion briefly and clearly to answer the purpose of the research. Conclusion is not the result of the research.

References:

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https://f1000researchdata.s3.amazonaws.com/linked/184593.Surpano_Edt-Siban_Fish_%28Nuraini%29.

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Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

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Referee Report 30 October 2017

doi:10.5256/f1000research.13967.r27095



Javad Ghasemzadeh 

Faculty of Marine Sciences, Chabahar Maritime University, Chabahar, Iran

This paper is the outcome of a well designed and executed research plan. It has clearly reviewed the current literature and background of the research, and the objectives of their research, and clear explanation of materials and methods which can be used and replicated in similar research work on different fishes. The statistical analysis applied for this type of research is simple, logical and interpreted very well. The results are given based on the clear and measured outcomes of the methods applied in the experiment which is applicable and reproducible in similar research work on the induced and artificial propagation of fishes (especially on freshwater fishes). The conclusions are clearly based on the measured and practically approved results, with no particular limitations being addressed. This article is good enough to be indexed with its present condition, and I did not find any problem in the English language used,.

Is the work clearly and accurately presented and does it cite the current literature?

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Are sufficient details of methods and analysis provided to allow replication by others?

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If applicable, is the statistical analysis and its interpretation appropriate?

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Are all the source data underlying the results available to ensure full reproducibility?

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