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Internal Fertilization of Oviparous Fishes, *Oryzias Celebensis*: Is It a Coincidence?

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Abstract: *Oryzias celebensis* is an oviparous endemic fish of Sulawesi. Compared to its Japanese counterpart (*Oryzias latipes*), this fish has not been studied much. The purpose of the article is to expand its study. The scientific novelty proves that internal fertilization in *Oryzias celebensis* is a part of reproductive strategy. It is not fortuitous. In the laboratory, we do routine work to breed female and male brood stock to produce embryos for ecotoxicology testing. We accidentally found an internally fertilized egg when we were stripping a female brood stock that had fertilized it externally two days earlier. These findings, combined with data on successful artificial insemination of *Oryzias latipes* and sperm motility of *O. latipes*, seem strong enough to reject the mainstream view that internal fertilization in *the Oryzias genus* is the facultative internal fertilization that occurs coincidentally. A new view of fertilization of *O. celebensis* will produce a new discourse in ecotoxicology studies.

Keywords: *Oryzias celebensis*, facultative internal fertilization, endemic, ecotoxicology.

卵生魚的體內受精，藜麥: 這是巧合嗎？

摘要: 藜麦是苏拉威西岛的一种卵生特有鱼类。与它的日本对应物（水稻）相比，这种鱼的研究并不多。这篇文章的目的是扩大其研究。科学新颖性证明藜麦的内部受精是繁殖策略的一部分。这不是偶然的。在实验室中，我们进行常规工作，以培育雌性和雄性亲本，以生产用于生态毒理学测试的胚胎。当我们剥离两天前外部受精的雌性亲鱼时，我们意外地发现了一个内部受精卵。这些发现，结合成功人工授精和哦. 扁豆精子活力的数据，似乎足以拒绝主流观点，即水稻属的内部受精是巧合发生的兼性内部受精。哦. 芹菜施肥的新观点将在生态毒理学研究中产生新的论述。

关键词： 藜麥，兼性內受精，地方性，生態毒理學。

1. Introduction

Oryzias celebensis is a fish endemic to Sulawesi from the order *Cyprinodontidae*. This fish is classified as an oviparous fish whose eggs develop outside the brood stock's body. Compared to *O. latipes*, O. *celebensis* has received less serious attention from researchers in the world. In Indonesia, especially in South Sulawesi, there are only 16 studies on *O. celebensis*, only some of which can be accessed via the internet, and some are stored in offline libraries as undergraduate or postgraduate theses. In addition, from the BASE (Bielefeld Academic Search Engine), we did not get many research papers on *O. celebensis* compared to *O. latipes*. We know from the BASE that we only get 38 papers hits in 272,588,409 documents using the keyword *Oryzias celebensis*. If we search for papers with keywords *Oryzias latipes*, a species distributed in Japan, China, Taiwan, and Korea [1], we

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will find 4,397 paper hits in 272,588,409 documents. Thus, we can conclude that research and publications on *Oryzias celebensis* are still very few compared to its partner *Oryzias latipes*. Although, we know that *O. celebensis* has great potential as a model fish as its partner *O. latipes* which has long been used as a model animal in various research fields, such as endocrinology and ecotoxicology oncology, etc. [2].

2. Methods

As an oviparous fish, *O. celebensis* is the same as its Japanese counterpart, *O. latipes*, as described by [3] that *O. latipes* is an oviparous fish with eggs that are not auto-active even though they are in ovarian fluid or seawater or tap water. The eggs will only be active after being penetrated by sperm. The eggs of the sea fish, *Alcichthys*, cannot be active even when they encounter sperm in ovarian fluid or hypotonic saline [3, 4]. It can only be active when exposed to seawater and encounters with sperm. In contrast, eggs are activated in *Salmon* and *Carasius auratus* when immersed in tap water or a hypotonic solution [3].

Oryzias eggs are spawned through a process of pseudocopulation with fertilization and development outside the body, where the eggs are placed in the abdomen. In 1931, [5] described his findings that *O. latipes* also performed internal fertilization in addition to its actual reproductive behavior, external fertilization. This finding occurred when [5] accidentally encountered a batch of *O. latipes* eggs with different developmental stages. Brood stock of *O. latipes* that had been mated ten days previously was dissected and was found in a batch of eggs, some of which had a more advanced developmental stage than others. This indicated clearly that during the copulation process, several sperm cells entered the ovarian cavity and fertilized mature eggs.

The same thing happened at *O. celebensis* in our laboratory. We routinely breed *O. celebensis* brood stock in a large container with four fish per liter density. Female brood stock that has undergone fertilization are separated into smaller containers and paired with male brood stocks ready to mate. *O. celebensis* males who are ready to mate have a special characteristic. Namely, the color pattern is brighter and pronounced. The color of the male brood stock anal fins that are ready to mate is blackened. Some are completely blackened, some are partly. Black and yellow stripes are more pronounced on the caudal fin (Fig. 1). After anesthetized the female brood stock using tricaine solution 0.17 mg/l for five minutes, we stripped the abdomen of a female brood stock that had been fertilized two days earlier. Of the 25 eggs we obtained from stripping, there were four active eggs. The active eggs were indicated by the formation of a formed perivitelline space (Fig. 2).

Fig. 1 The male fish that ready to mate

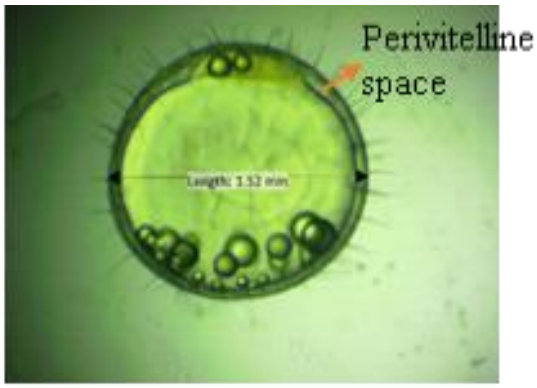

Fig. 2 Internally fertilized eggs

3. Results

Upon observation, it seemed that the eggs could not develop until the end of the stage and hatch due to death. [5] observed that the embryo resulting from internal fertilization provides a clear picture. The optic vesicle and tail were formed, the heartbeat and the yolk circulated continuously. The perivitelline space of the embryo was slightly narrower than that of normal embryos that developed outside the brood stock body. Pigment cells were found scattered throughout the embryo. However, these embryos died a few days before hatching [5]. [6] stated that the phenomenon of internal reproduction in *O. latipes* is the facultative internal fertilization, which results from the proximity of male and female gonophores during fertilization. [6] also stated that it is fortuitous internal fertilization. [5] suggested internal fertilization transition from oviparity to viviparity.

What has been discovered by [5] was elaborated by [7] to determine whether there was an effect of ovary fluid on survival and sex differentiation by artificial insemination. Female of *O. latipes* brood stock was injected with male brood stock sperm. After 48 and 72 hours, the eggs were removed from the female brood stock's ovary cavity and placed on the rearing medium until they hatched. The results showed that the embryo developed perfectly, and there was no effect of maternal steroids on sex differentiation.

With the success of [7] in performing artificial insemination on *O. latipes*, the question arises whether it is true that internal fertilization performed by *O. latipes* and *O. celebensis* is a fortuitous reproductive strategy as mentioned by [6]. It should be noted that stating a phenomenon in the empirical world as something fortuitous according to [8] is the expression of people who do not find causality; it is not an argument based on valid arguments. Alternatively, is it a

constructive reproductive strategy adopted by *O. latipes* and *O. celebensis* due to the K strategy? If internal fertilization is not a coincidence, then what is the rational reason for the process? Do other species of Oryzias in Indonesia, such as *O. woworae*, *O. wolasi*, etc., also undergo internal fertilization?

4. Discussion

The internal fertilization carried out by *O. latipes* and *O. celebensis* was not coincidental or fortuitous. This is supported by the fact that the sperm motility of *O. latipes* can remain up to one week [9], whereas in general, in fish that perform external fertilization, sperm motility has a short time a few seconds after release from the urogenital opening. By looking at the ability of sperm motility to survive for a long enough time, and supported by the pseudocopulation behavior with the proximity of female and male gonopore during copulation, internal fertilization is not fortuitous in genus *Oryzias*. However, it could be a constructive reproductive strategy following the K strategy. Fertilization of eggs in the ovarian cavity of the female brood stock appears to be a backup for externally fertilized eggs, which are more susceptible to predatory activity. Animals with the K strategy must ensure that their offspring's safety conditions are guaranteed [10-16]. It is due to the embryo produced by animals using the K strategy, not as much as those produced by animals using the R strategy [13, 14, 17, 18]. In addition, the K strategy fish invests much energy in the embryo or offspring, and conversely, the R strategy fish invests less energy in the resulting embryo or offspring [19]. In our laboratory, *O. celebensis* produces 30 eggs on average for one fertilization. This amount is very small compared to the number of fish eggs using the R strategy such as *Cyprinus carpio* [20-22].

[23] observed that the estimated amount of sperm released per spawning of *O. latipes* was correlated positively and significantly with morphometry-paired females. The observed behavior follows the sperm allocation hypothesis, which states that the male *medaka* allocates his sperm based on the morphology of the paired female. This fact also confirms the hypothesis that internal fertilization of *O. celebensis* is a planned and therefore a reproductive strategy to ensure that the offspring can spawn successfully.

In addition, the data observed by [23] clearly showed that male *O. latipes* did not arbitrarily release sperm but did so with careful calculations based on the morphology of the paired female. Moreover, according to [24], Jellyfishes and bryozoans fertilize internally without mating. The male brood stock releases his sperm into the waters, and the sperms swim towards the female brood stock's body and find the eggs, and fertilization occurs [24]. Thus, internal fertilization in the genus *Oryzias* cannot be stated as fortuitous fertilization, but it is a reproductive strategy that is innate in its evolutionary process.

However, the hypothesis that internal fertilization is not fortuitous needs to be studied more deeply to obtain a solid scientific justification. *In vivo* and *situ,* research needs to be done on internal fertilization in *Oryzias celebensis* and several species of paddy fish distributed in Indonesia. In addition, since the genus *Oryzias* is a sentinel organism or model fish for ecotoxicological testing both in the laboratory and in fields [25-30], it is necessary to know the Darwinian fitness of embryos produced by internal fertilization against pollutants.

5. Conclusion

The finding on internal fertilization in the oviparous genus *Oryzias* which are supported by several other facts from several studies such as artificial insemination studies, sperm motility of *Oryzias* which can survive up to one week in water, and sperm allocation by males at spawning based on the body size of female brood stocks seems to be quite strong as evidence to refute the common view that the internal fertilization in Oryzias genus is facultative fertilization that occurs by chance. This is the scientific novelty of this paper, which shows that internal fertilization in *Oryzias latipes* and *O celebensis* is a reproductive strategy constructively as a backup from external fertilization, common in oviparous fish. The finding described in this paper still needs to be elaborated in more constructive and systematic studies because there are several limitations in these findings, such as embryos that cannot survive until hatching. In addition, the phenomenon of internal fertilization needs to be investigated in all paddy fish, especially those from the *Oryzias* genus, which are distributed in Indonesia, especially from the island of Sulawesi. From an ecotoxicological perspective, it is necessary to study contaminants that can stimulate internal fertilization and how the Darwinian fitness of the embryos resulting from internal fertilization is exposed to pollutants.

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