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# The Cost and Effectiveness of CPH, HCG and LHRH-a on the Induced Spawning of Grey Mullet, *Mugil cephalus*

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ABSTRACT

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Two hormone injections, a primer and a resolver, were used to induce spawning in grey mullet. Combinations of carp pituitary homogenate (CPH), human chorionic gonadotropin (HCG) and luteinizing hormone-releasing hormone analogue (LHRH-a), each used as a primer or resolver, were tested in 60 spawning attempts. Another six fish received saline injections and served as sham controls. The cost and the response, in terms of spawning and fertilization rates, of each combination were evaluated. The CPH/LHRH-a treatment is the most reliable and cost-effective method. HCG can replace CPH in this treatment, but the cost is higher and the response poorer. The HCG/LHRH-a treatment can, however, be used for research purposes.

## INTRODUCTION

Natural spawning of grey mullet (*Mugil cephalus*) in captivity has not yet been demonstrated. Hormonal intervention is presently the most effective method for inducing final maturation and successful spawning. Hormones used to induce spawning in mullet include fish pituitary homogenates from mullet, salmon and carp (Tang, 1964; Yashouv et al., 1969; Shehadeh and Ellis, 1970; Shehadeh et al., 1973), steroids (Kuo and Nash, 1975; Kuo and Watanabe, 1978), HCG (Kuo et al., 1973), LHRH-a (Lee et al., 1987), a combination of pituitary homogenate with synahorin (Liao, 1969; Shehadeh and Ellis, 1970), a combination of carp pituitary homogenate and LHRH-a (Lee et al., 1987), and others (see reviews by Nash and Shehadeh, 1980; Kuo, 1982).

The most reliable method for spawning mullet is an acute hormonal therapy combining either carp pituitary homogenate (CPH) with human chorionic gonadotropin (HCG) or CPH with luteinizing hormone-releasing hormone analogue (LHRH-a). In both cases, CPH is given as the priming dose. There

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are at present no data on reversing the injection order. A major drawback to the use of CPH is inconsistency in its potency, which makes dosage comparisons among research groups futile. Kuo et al. (1973) spawned mullet with a hormonal strategy of HCG/HCG. One of the advantages of using HCG is that the potency is standardized in international units and results can be compared among users. The dosage required for inducing mullet to spawn is relatively high, however, and too expensive to be economically feasible for farming practice.

In the present study, CPH, HCG and LHRH-a were tested in various combinations. The rate of spawning success and costs related to the different hormonal therapies were compared.

## MATERIALS AND METHODS

Mature females were selected from the broodstock holding facilities at the Oceanic Institute. Selection and induced spawning trials took place in 1986 and 1987 during the natural spawning season in Hawaii (end of December to mid-March). The broodstocks were held in outdoor, rectangular, rubber-lined ponds or in outdoor, round, concrete tanks. The fish fed on naturally occurring benthic growth and were given supplemental Purina Trout Chow. Water temperature and salinity ranged from 27°C and 32‰ to 32°C and 38‰ throughout the spawning season.

The criterion for selection of females for the experiments was possession of tertiary yolk globule stage eggs that averaged 600 μm or larger. Attainment of this egg diameter has been established by Kuo et al. (1973) and Shehadeh et al. (1973) as being necessary for successful induction of spawning. The chosen females were placed in a 170-liter spawning tank and given one of the seven hormone treatments studied (Table 1). Each treatment consisted of priming and resolving injections. The seven treatments tested were saline injection used as a sham control, CPH/CPH, LHRH-a/LHRH-a, CPH/LHRH-a, LHRH-a/CPH, HCG/LHRH-a and LHRH-a/HCG. In most cases these experiments were run in parallel to avoid seasonal differences in response.

CPH was purchased from Argent Chemical Laboratory, Washington, U.S.A., HCG from Sigma Chemical Company, Missouri, U.S.A. and LHRH-a from Oriental Scientific Instruments Import and Export Corporation Shanghai, China. The form of LHRH-a used in these experiments was [D-Ala$^6$, Pro$^9$ NHEt] LHRH.

Dosages of CPH, HCG and LHRH-a used in the present study were based on results of previous investigations (Kuo et al., 1973; Shehadeh et al., 1973; Lee et al., 1987) (Table 1). The priming and resolving injections were given 24 h apart, between 08.00 and 10.00 h. After each female was given the resolving injection, two or three males were introduced into the spawning tank with her. After spawning was achieved, fertilization rates were checked, spawned eggs were collected and incubated in 1000-liter, open-system incubators. For each spawn, the time of spawning, the total numbers of eggs, egg diameters, fertilization rate, and hatching rate were recorded. The spawning rate was calculated as the percentage of females which were induced to spawn with their respective therapies.

The production cost was estimated in order to compare the efficiency of different hormone therapies. The estimated hormone cost of producing mullet larvae was based on price of hormones, results of induced spawning attempts and the following assumptions. First, there was no difference in maturational condition of males among the treatments. The fertilization rate is based solely on the condition of the females. Second, the average fecundity of a 1-kg female is 1 000 000 eggs. Third, the hatch rate is 50%. Fourth, the cost for support facilities and labor are constant among treatments. These costs were not calculated, although they cannot be ignored in the calculation of the total production cost. Therefore, the cost of hormone per 100 000 larvae produced =

$$\frac{\text{cost of hormone} \times 100\,000}{\text{fecundity} \times \text{spawning rate} \times \text{average fertilization rate} \times \text{hatching rate}}$$

The potential number of fertilized eggs from one female was calculated by multiplying the fecundity by spawning and fertilization rates of a particular therapy. The number of hatched larvae is the product of the potential number of fertilized eggs and the hatch rate.

## RESULTS

None of the mullet given two saline injections spawned (Table 1). The spawning success rate with the other therapies, however, ranged from 75 to 100%. The only therapy which did not result in spawning when the two-injection protocol was used was LHRH-a/HCG. Two of the females given this treatment did spawn, however, when an additional injection of LHRH-a was administered. These two females had received the higher dosage of HCG (10 000 IU) for their second injection.

The CPH/LHRH-a therapy yielded significantly higher fertilization rates than all the other treatments ($P < 0.001$, $t$-test, Table 1). All other therapies exhibited wide fluctuations in fertilization rates and did not differ significantly from each other ($P > 0.10$).

The interval between the second injection and the time of spawning was noticeably longer when the LHRH-a/LHRH-a therapy was used. All other treatments resulted in a relatively consistent time interval.

The cost of the hormones used to induce mullet to spawn is presented in Table 1. The price ranges reflect differences between suppliers. In general, the use of HCG exerts the greatest influence on the cost of just the hormones which are used to induce mullet to spawn.

TABLE 1

Dosages and cost of hormones for induced spawning of mullet using different strategies

| Hormone treatment | Dosage | Cost (US$) | Spawning rate (%) | Fertilization rate (%)[c] | Time of spawning after 2nd injection[c] (h:min) |
|---|---|---|---|---|---|
| Saline | | | 0 (6)[a] | | |
| CPH/CPH | 20 mg/40 mg | 8.40 | 75 (8) | 49.7 ± 17.8<br>23.6 – 73.0 | 12:18<br>10:00 – 15:20 |
| CPH/LHRH-a | 20 mg/200 μg | 3.81 – 6.95 | 90 (20) | 86.9 ± 9.0<br>66.0 – 100 | 12:42<br>10:36 – 17:30 |
| LHRH-a/CPH | 200 μg/20 mg | 3.81 – 6.95 | 100 (5) | 49.9 ± 29.9<br>7.0 – 87.0 | 11:12<br>10:00 – 12:33 |
| LHRH-a/LHRH-a | 400 μg | 2.02 – 8.30 | 87.5 (8) | 46.8 ± 44.1<br>0 – 97.9 | 17:26<br>11:48 – 21:00 |
| HCG/LHRH-a | 5000 IU/200 μg | 7.56 – 10.70 | 100 (5) | 53.6 ± 34.8<br>0 – 97.2 | 14:24<br>11:28 – 17:55 |
| HCG/LHRH-a | 10 000 IU/200 μg | 14.11 – 17.25 | 83.3 (6) | 65.8 ± 19.1<br>40.1 – 97.0 | 12:50<br>11:00 – 14:40 |
| LHRH-a/HCG | 200 μg/5000 IU | 7.56 – 10.70 | 0 (4) | – | – |
| LHRH-a/HCG | 200 μg/10 000 IU | 14.11 – 17.25 | 0 (2)[b] | – | – |

[a]Number of fish tested.
[b]Fish spawned after additional LHRH-a injection.
[c]Values in second row are range.

TABLE 2

Estimated production cost of mullet larvae in the hatchery

| Treatment | Fertilized eggs | Number of hatched larvae | Hormone cost per 100 000 larvae produced (US$) |
|---|---|---|---|
| CPH/CPH | 372 750 | 186 375 | 4.51 |
| CPH/LHRH-a | 782 370 | 391 185 | 0.97-1.78 |
| LHRH-a/CPH | 499 000 | 249 500 | 1.53-2.79 |
| LHRH-a/LHRH-a | 409 500 | 204 750 | 0.99-4.05 |
| 5000 IU HCG/LHRH-a | 536 000 | 268 000 | 2.82-3.98 |
| 10000 IU HCG/LHRH-a | 548 114 | 274 057 | 5.16-6.29 |

Table 2 compares the cost of producing mullet larvae based solely on the price of the hormones used. The results indicate that CPH/LHRH-a was the most cost-effective method and HCG/LHRH-a (using 10 000 IU HCG) was the least cost-effective method for producing larvae. Production using LHRH-a/LHRH-a varied by up to 400% depending on the unit price of LHRH-a available from different sources.

## DISCUSSION

At the present time mullet will not spawn in captivity without hormonal intervention. Kuo et al. (1973) concluded that mullet will spawn within 24 h after a priming injection of 20 IU of HCG/g body weight and a resolving injection of 40 IU of HCG/g. This method is both simple and convenient. The cost to spawn a 1-kg fish using this method, however, is US$78.60 using the least expensive HCG available. In addition, this gonadotropin requires storage at low temperatures (0–5 C) which makes use in the field more difficult. Researchers at the Oceanic Institute recently replaced the priming dose of HCG with CPH. The fish was induced to spawn using 40 mg CPH and 40 000 IU HCG. The cost was still prohibitive at US$57.20 for a 1-kg fish. Although this method is reliable, it is too expensive and was not considered in this study. The cost per fish can be reduced to US$7.37 by injecting 50 mg CPH and 100 mg of a steroid such as deoxycorticosterone (DOC) (Kuo, 1982). The application of steroid hormones to spawning, however, requires further study because the DOC solution is difficult to prepare and results are still inconsistent. For these reasons, this method was also not considered in this study.

The prices of HCG and LHRH-a vary by company and also when the hormone is purchased in bulk. Based on the sources we used in this study, LHRH-a/LHRH-a and CPH/LHRH-a are the least expensive and HCG/LHRH-a is the most expensive of the hormone treatments we tested. The cost for either LHRH-a/LHRH-a or CPH/LHRH-a is less than 15% of the cost of CPH/HCG treatment.

The induced spawning method which uses a single injection of LHRH-a has been used to spawn a number of carp species in China. It has not been well received, however, because results are inconsistent and spawning time is unpredictable (Peter et al., 1988). Similar kinds of results were obtained with the application of LHRH-a alone for both the priming and resolving injections in inducing mullet to spawn. Only moderate spawning success and fertilization rates were achieved using this form of therapy (present study and Lee et al., 1987). A dramatic improvement resulted by replacing LHRH-a with CPH as the priming injection. In a similar study the CPH/LHRH-a injection strategy was also found to provide good and consistent results in mullet (Lee et al., 1987).

When the CPH/LHRH-a hormone strategy was reversed, i.e., LHRH-a/

CPH, spawning was induced, but a satisfactory fertilization rate (greater than 80%) was not consistently achieved. A gonadotropin preparation is hypothesized as being required as a priming injection to stimulate oocyte development from the tertiary yolk globule stage to the sub-peripheral germinal vesicle stage (Kuo, 1982). Application of gonadotropin-releasing hormone (GnRH) alone might not stimulate sufficient endogenous GtH secretion for oocyte development. Therefore, the spawning and fertilization rates achieved with LHRH-a/CPH and LHRH-a/LHRH-a were not high.

Although CPH shows the most promise, it has shortcomings. Unlike LHRH-a, the preparation of CPH relies on biological resources and its quality is not consistent among different sources. In addition, CPH sometimes produces lesions at the injection site in the mullet, causing mortality (unpublished data). In order to facilitate comparison among research groups, CPH should be replaced with a standardized gonadotropin preparation. HCG was selected as a replacement for CPH as a source of gonadotropin. The results showed that using either 5000 or 10 000 IU HCG to replace 20 mg CPH produced a higher rate of spawning success. The 5000 IU HCG treatment showed a wide range of fertilization rates compared with the 10 000 IU HCG treatment group. An increased dose of HCG may have produced a better fertilization rate. A priming dose of 16 700 IU HCG for HCG/HCG therapy was recommended by Kuo (1982). This may be the most appropriate dose for the HCG/LHRH-a method if cost was not a consideration. The cost of 10 000 IU of HCG, however, is more than four times the cost of 20 mg CPH. Therefore, dosages greater than 10 000 IU HCG were not tested in this study.

In the current study, CPH was shown to be a better spawning agent than HCG at the dosages tested. Fontaine (1976) also indicated that crude pituitary extract was more potent than purified GtH for inducing spawning. However, 10 000 IU HCG is still recommended as a replacement for CPH as a priming dose for research purposes because HCG can be standardized. The reciprocal therapy, LHRH-a/HCG, did not induce the mullet to spawn. The HCG dose used in LHRH-a/HCG may be too low to induce spawning. In the standard HCG/HCG hormone strategy, the resolving injection is between 33 300 and 40 000 IU/kg. The spawning success of the LHRH-a/HCG may improve with an increase in the dosage of HCG. Trials will be conducted to verify this hypothesis.

The comparisons in Table 2 are based on several assumptions. One of these is that fertilization rates depend only on the female. It is understood that fertilization rates also depend on the condition of males. The state of maturity for all males was assessed prior to use in spawning trials and only running ripe individuals were used. In addition, a 3:1 male to female sex ratio was used in all of our spawning trials to optimize fertilization rates. Other assumptions on fecundity and hatch rate rely on a previous investigation (Nash and Shehadeh, 1980). The authors believe that these are reasonable assumptions and that results pertaining to the cost efficiency of the various hormonal regimes tested are valid.

In conclusion, the CPH/LHRH-a treatment was the best among those tested in terms of cost and effectiveness. CPH, in the CPH/LHRH-a strategy, can be replaced by a gonadotropin preparation such as HCG. The HCG/LHRH-a treatment was expensive and results were inferior to the CPH/LHRH-a treatment. It is a desired treatment, however, for comparison among research groups because dosages of HCG and LHRH-a can be quantified.

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REFERENCES

Fontaine, M., 1976. Hormones and the control of reproduction in aquaculture. J. Fish. Res. Board Can., 33: 922–939.

Kuo, C.M., 1982. Induced breeding of grey mullet, *Mugil cephalus* L. In: C.J.J. Richter and M.J.Th. Goos (Compilers), Proc. Int. Symp. Reproductive Physiology of Fish, Wageningen, The Netherlands. PUDOC, Wageningen, pp. 181–184.

Kuo, C.M. and Nash, C.E., 1975. Recent progress on the control of ovarian development and induced spawning of the grey mullet (*Mugil cephalus* L.). Aquaculture, 5: 19–29.

Kuo, C.M. and Watanabe, W.O., 1978. Circadian responses of teleostean oocytes to gonadotropins and prostaglandins determined by cyclic AMP concentration. Ann. Biol. Anim. Biochem. Biophys., 18(4): 949–956.

Kuo, C.M., Nash, C.E. and Shehadeh, Z.H., 1973. Induced spawning of captive grey mullet (*Mugil cephalus* L.) females by injection of human chorionic gonadotropin. Aquaculture, 1: 429–432.

Lee, C.-S., Tamaru, C.S., Miyamoto, G.T. and Kelley, C.D., 1987. Induced spawning of grey mullet (*Mugil cephalus*) by LHRH-a. Aquaculture, 62: 327–336.

Liao, I.C., 1969. Artificial propagation of grey mullet (*Mugil cephalus* Linnaeus). Fish. Ser. Chin.-Am. Joint Comm. Rur. Reconstr., 8: 10–20.

Nash, C.E. and Shehadeh, Z.H., 1980. Review of breeding and propagation techniques for grey mullet, *Mugil cephalus* L. ICLARM Studies and Reviews No. 3. Int. Cent. Living Aquatic Resources Management, Manilla, 87 pp.

Peter, R.E., Lin, H.-R. and Van Der Kraak, G., 1988. Induced ovulation and spawning of cultured freshwater fish in China: advances in application of GnRH analogues and dopamine antagonists. Aquaculture, 74: 1–10.

Shehadeh, Z.H. and Ellis, J.N., 1970. Induced spawning of the striped mullet *Mugil cephalus* L. J. Fish Biol., 2: 355–360.

Shehadeh, Z.H., Kuo, C.M. and Milisen, K.K., 1973. Induced spawning of grey mullet *Mugil cephalus* L. with fractionated salmon pituitary extract. J. Fish Biol., 5: 471–478.

Tang, Y.A., 1964. Induced spawning of striped mullet by hormone injection. Jpn. J. Ichthyol., 12(1/2): 23–28.

Yashouv, A., Berner-Samsonov, E. and Karamosta, K., 1969. Preliminary report on induced spawning of *M. cephalus* (L.) reared in captivity in freshwater ponds. Bamidgeh, 21: 19–24.