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PROVIDING LARVAL FISH AS FOOD FOR LARVAE OF SKIPJACK TUNA: INVESTIGATIONS ON INDUCED SPAWNING OF MANINI, OR CONVICT SURGEONFISH (Acanthurus triostegus sandvicensis).

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This report was prepared under contract (83-JJA-00347) by Calvin M. Kaya. The main objectives of this study were to develop suitable methods for inducing spawning in the convict surgeonfish (Acanthurus triostegus sandvicensis) and to test the suitability of the resulting larvae as a food fish for larval and juvenile tuna. Since this report was prepared under contract, the statements, findings, and conclusions herein are those of the contractor and do not necessarily reflect the view of the National Marine Fisheries Service.

Richard W. Brill
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November 29, 1983

Preface

It was mutually agreed between myself and Dr. Richard Brill, in charge of experimental investigations at the Kewalo Research Facility, that the original objectives of this study program should be modified to include and emphasize the development of techniques to produce, on demand, fish larvae of a non-tuna species. Such larvae could then be available to feed to tuna larvae as part of the ongoing program to rear young tuna in captivity at the Kewalo Research Facility. The original study included two aspects: (1) experimental determination of optimum temperatures and upper and lower median thermal tolerance limits for embryos and prolarvae of skipjack tuna; and (2) investigation of a possible mechanism for "stress-induced spawning" of skipjack tuna. In the revised study program, efforts to induce spawning of a non-tuna species replaced the proposed work on stress-induced spawning and was assigned primary emphasis. The experiments on temperature relations of embryos and prolarvae were to be retained but given secondary priority.

The experimental apparatus for maintaining embryos and prolarvae at six different temperatures was set up and its functioning tested and confirmed ready for initiation of these investigations. However, this part of the study did not progress beyond this. The Kewalo Research Facility was not able to acquire live adult tuna to spawn and produce the fertilized eggs to commence these experiments, within the 30-day contracted period or within an additional two weeks that I made myself available.

The following report, therefore, will include only the work on induced spawning of a non-tuna species, the manini or convict surgeonfish.

Providing Larval Fish as Food for Larvae of Tuna: Investigations on Induced Spawning of Manini, or Convict Surgeonfish (Acanthurus triostegus sandvicensis).

Introduction

The purpose of this study was to attempt to develop techniques to artificially induce spawning of manini at the Kewalo Research Facility and thereby to provide, on demand, fish larvae as food for young skipjack tuna being reared in captivity. Larvae of these tuna have been hatched out and reared at the Kewalo Research Facility up to an age of two weeks posthatch. However, larvae from one batch of eggs sent to the La Jolla Laboratory were reared beyond two weeks and one specimen lived for 35 days before dying of accidental causes. Although the reasons for this difference in survival have not been determined, it is suspected that an important factor was the feeding of fish larvae (halibut) to the skipjack larvae reared at La Jolla. Investigators at both laboratories fed the same species of cultured rotifers and copepods to the tuna larvae, but fish larvae of the appropriate size for this purpose have not been available at the Kewalo Research Facility.

Manini were selected for this study for several reasons. One is that they are very easily maintained in the large holding tanks at this laboratory, where they are kept to help control growths of algae on the walls and floors of these tanks. Another is that they are known to mature sexually in captivity and have been occasionally observed to spawn naturally in these tanks. Finally, the observations of Randall (1961) indicate that the larvae are small enough (yolk-sac larvae 1.7 mm in total length) to be ingested by skipjack tuna larvae less than two weeks of age.

Methods

Specimens for these studies were adult manini maintained in captivity at the Kewalo Research Facility for long, undetermined periods of up to several years. Techniques were developed to identify the sexes from external characteristics (as described in the Results section). For each of two experiments, one male and two females were isolated in a circular experimental tank 4 ft (1.23 m) in diameter and 18 inches (45.7 cm) in depth. Because the males could be stripped of milt before being placed into the experimental tank, they were not treated with hormones. Females received varying dosages of human chorionic gonadotropin (HCG) alone, or HCG and powdered salmon pituitaries dissolved or suspended in 1.1% NaCl solution. Injection volumes were 0.1 or 0.2 ml, depending on dosage level. Treatments are summarized in Table 1. Each female was injected twice, the first time at 9:30 to 10:30 am and the second 24 hours later. A fine-meshed net was placed over the outflow from the experimental tank to detect any release of ova by the treated females. Specimens were 180 to 209 mm in fork length and 176 to 229 g in weight. The experimental tank received continuous flow of seawater and temperatures in the tank ranged from about 25 to 28 C.

At varying intervals following the second injection, as described in the Results section, females were tested for ovulation by application of stripping pressure to the abdomen. If ripe ova were produced, they were stripped into a glass bowl and fertilized by stripping milt from the male present in the tank. Fertilization was thus effected by the "dry method" and the eggs and milt gently stirred in the receptacle for 3 to 5 minutes. In both experiments 1 and 2, the eggs were placed in 3-liter beakers filled with filtered seawater and provided with aeration. In the second

experiment, most of the eggs were transfered to 16-liter plastic buckets filled with filtered seawater and aerated. Also in the second experiment, 1000 advanced embryos were transfered to a 100-liter culture tank provided with filtered seawater, aerated, and inoculated with 4 liters each of two cultured algae, Tetraselmis sp. and Isochrisis sp. After hatching of the larvae, cultured rotifers, Brachionus plicatilis were also introduced into the culture tank and these rapidly increased into a dense population by the third day posthatch. Starting on the third day posthatch, 40% (40 liters) of the water volume was replaced with fresh, filtered seawater. Daily additions of 4 liters of each of the cultured algae were also added to replenish comsumption by the rotifers.

Results

In manini, sexes can be distinguished by examination of the ventral slit in which the anus, genital pore and urinary pore are located. In males this slit is narrower and the urogenital area often cannot be seen unless pressure is applied to the abdomen to produce slight protrusion of this area. The ventral slit is wider in females and the urogenital area can be seen more readily. Also, there is a transverse fold readily visible between the anus and the genital area of females, but not of males. As females approach spawning condition the genital area becomes swollen and expands the width of the ventral slit. Ripe males will yield milt in response to light to moderate stripping pressure, making them particularly easy to identify during the breeding season.

Responses of the females to hormone treatments are summarized in Table 1. Ovulation was induced in three of the four treated females, both fish of Experiment 1 and one of the two in Experiment 2. Subsequent dissection of the single unresponsive female indicated that its ovaries were immature,

containing only small ova up to about .06 to .07 mm in diameter. Combinations of HCG and salmon pituitaries or HCG alone were effective in stimulating ovulation. The greatest response was from the female treated with the highest dosage of HCG, used alone without salmon pituitaries (Female 2A).

None of the three responsive females released ova externally and ovulation was confirmed by manually stripping ova from each. In the first experiment the treated fish were left undisturbed following each injection in order to provide the opportunity for them to spawn with the male present in the tank. When this did not occur, both females were tested with stripping pressure applied 25 hours after the second injections. Ovulation was confirmed by the release of ripe ova from each. In the second experiment the females were tested with stripping pressure applied 4, 8, 10, and 12 hours following the second injections. One female, as previously mentioned, did not ovulate. The other female did not yield ova after 4 hours postinjection but released an estimated 49,500 after 8 hours and an estimated 33,250 after 10 hours postinjection. Only a small number of ova were released after 12 hours postinjection and this part of the experiment was terminated at that time.

Ova stripped from the responsive females in each experiment were fertilized with milt stripped from the male present in the experimental tank. With the ova from the first experiment, stripped from the two females about 25 hours following the second injections, there was no evidence of successful fertilization and no embryonic development observed. With the ova from the second experiment, initial fertilization rates were not determined but after 19 to 23 hours, about 23% of the ova stripped out after 8 hours and about 40% of the ova stripped out after 10 hours following the second injection were undergoing advanced embryonic development.

Table 1. Responses of female manini to two daily injections of human chorionic gonadotropin (HCG), or HCG and powdered salmon pituitaries (SP).

Spec. No.	1st Injection	2nd Injection	Response
		Experiment 1	
1A	100 IU HCG	100 IU HCG + 1.0 mg SP	Estimated 16,000 ova stripped about 25 hours after second injection
1B	100 IU HCG	100 IU HCG + 1.0 mg SP	Estimated 1,700 ova stripped about 25 hours after second injection
		Experiment 2	
2A	200 IU HCG	500 IU HCG	Estimated total of 83,000 ova stripped in two batches, 8 and 10 hours after second injection
2B	300 IU HCG	600 IU HCG + 20 mg SP	no ovulation, female with immature ovaries

Embryonic and early larval development followed the pattern and approximate temporal sequence described by Randall (1961). Ripe eggs were about .65 to .70 mm in diameter and those fertilized were slightly bouyant and floated in unagitated seawater. Nonviable eggs either sank or also floated. At incubation temperatures of about 24.5 to 28 C, the first cleavage divisions were evident by one hour after fertilization. The larvae hatched out during the second night. Times of hatching were not observed, but Randall (1961) reported that manini larvae hatched out about 26 hours after fertilization at incubation temperature of about 24 C. The number of larvae hatched out was estimated to be about 17,000 not including those in the culture tank.

The larvae in the two 16-liter plastic buckets were not fed. Their survival appeared very good until the third day, when large numbers appeared to by dying by midafternoon. Numbers of larvae were greatly reduced by the fourth day and few could be found on the fifth day after hatching.

The larvae in the 100-liter culture tank started feeding on the third day after hatching. Clumps of algal cells were clearly visible in their digestive tracts and they appeared healthy and active at this time. This same pattern continued through the fourth day posthatch, but numbers of larvae in the culture were considereably diminished by the morning of the fifth day. On the sixth day, the remaining larvae were obviously dying, no longer swimming actively but instead mostly drifting passively with currents induced by aeration of the tank. On the seventh day posthatch, live larvae could no longer be found. Larvae fed on algae from the third through the sixth day, but I could not see any rotifers in their digestive tracts despite the high densities of both adult and immature Brachionus in the culture tank.

Discussion

The results indicate that HCG used alone is an effective ovulating agent for manini. This is advantageous since HCG is readily available, easy to work with, and relatively inexpensive. The much greater response by female 2A than by either females 1A or 1B suggests that the higher dosages of HCG administered to the former were a more effective treatment. However, this is a tentative conclusion based on responses by only three specimens. Further experimentation was made impractical by the accidental deaths, associated with an electrical power failure, of 14 of the 24

adult specimens remaining after the second experiment. Since the treatment administered to female 2A was highly effective in producing viable,fertilizable ova, it was decided that the surviving adult specimens should be saved to produce manini larvae for the tuna larval rearing trials.

The complete infertility of the ova stripped from females 1A and 1B 25 hours following the second daily injection, and the difference in fertility between the two batches of ova stripped from female 2A indicate that viability of the eggs decreases rapidly following ovulation. With female 2A, it was apparent that large numbers of ova had been ovulated by 8 hours following the second injection. It is likely that many of the ova stripped out at this time had been ovulated more than an hour earlier. It also appeared that ovulation was still continuing when the female was stripped at 8 hours, and that those stripped out two hours later, at 10 hours postinjection, therefore contained a higher proportion of recently ovulated eggs. This could have produced the difference in fertility (23% vs. 40% of eggs producing advanced embryos).

Although the larvae which were fed lived for two to three days longer than those not offered any food, it is not known to what extent the ingestion of algae, but seemingly not of rotifers, actually contributed to the longer survival. Other factors, such as the higher densities of larvae in the buckets than in the culture tank, daily replacement of 40% of the water in the culture tank but no water replacement in the buckets, and the possible effect of the algae on water quality in the culture tank, may have contributed to the difference in survival time.

Of the 14 manini killed during the electrical power failure, 8 were females. Of these, three had immature ovaries with largest ova 0.10 to

0.12 mm in diameter. The other five females had maturing ovaries with largest ova measuring about 0.38 to 0.42 mm in diameter. The ovaries of the latter almost certainly represented the starting condition of the ovaries of the three females which did respond to hormone treatments. The ovaries of the single unresponsive female were similar to those of the three accidentally killed females with immature ovaries. Randall (1961) found that the largest ova in mature female manini caught in Hawaiian waters during their breeding season (early December to late July in his study) have modal diameters of 0.35 to 0.42 mm. He found larger ova only in running ripe females and concluded that ripening eggs increase rapidly in size just before spawning. The proportion of mature females found by Randall (46 out of 54 examined) and among the captive specimens in this study (8 of 12, including both those accidentally killed and the experimental specimens), indicate that females responsive to hormone-induced spawnings should be readily available during the breeding season in either wild or captive populations. The use of two or more females in a given spawning attempt should make it highly probable that at least one will be sufficiently developed to respond.

Conclusions and Recommendations

Manini appear to represent a very promising potential source of larval fish as food for larval tuna. Juveniles and adults are maintained very easily and in apparent good health in the large holding tanks at the Kewalo Research Facility. The captive adults develop mature gonads and occasionally spawn naturally in these tanks.

Results of the present study indicate that mature adults maintained in captivity can be easily induced to spawn with injections of HCG. This hormone preparation is easy to administer, readily available, and relatively inexpensive. Yolk sac larvae will be available on the third

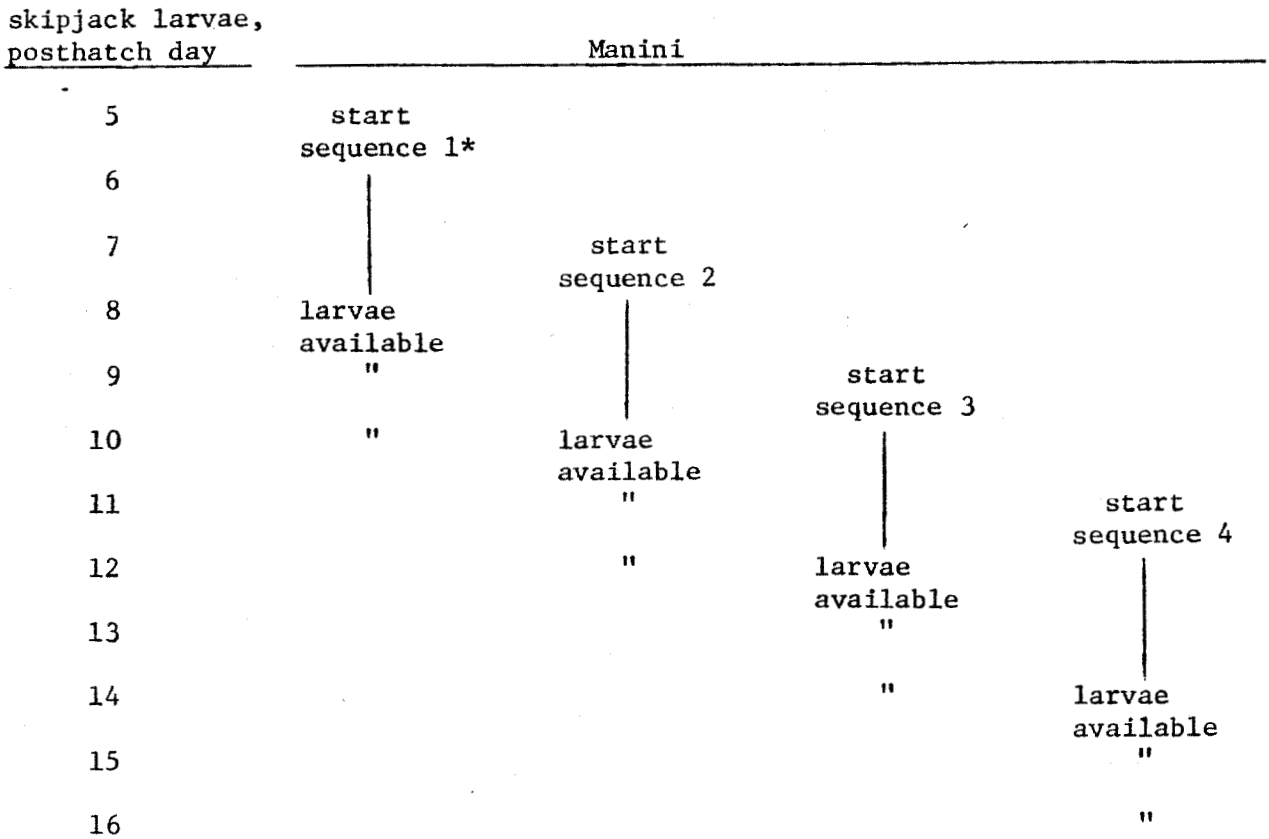
morning (about 72 hours) following the initiation of hormone treatments. Without any care other than being maintained in sufficient volume of aerated seawater, the yolk sac larvae and early prolarvae will remain alive and be available as food until the third day.

A recommended procedure and schedule for producing manini larvae for feeding to skipjack tuna larvae is presented in Appendix 1. The schedule assumes that skipjack tuna larvae are probably able to start feeding on fish larvae of this size by about their 8th to 10th day after hatching (T. Kazama and S. Hendrix, National Marine Fisheries Service, Honolulu Laboratory, pers. comm.)

Literature Cited

Randall, J. E. 1961. A contribution to the biology of the convict surgeonfish of the Hawaiian Islands, Acanthurus triostegus sandvicensis. Pacific Sci., 15: 215-272.

Appendix 1. Suggested procedure and schedule for producing manini larvae as food for larvae of skipjack tuna larvae.



* Each manini sequence with the following schedule:

first day: 9:00 to 9:30 am, inject each of two or more females with 200 IU HCG, intramuscular, in 0.1 ml saline solution (200 IU/0.1 ml)

second day: 9:00 to 9:30 am, inject each female with 500 IU HCG in 0.25 ml (same preparation as previous day, kept refrigerated)

starting at 3:00 pm (six hours postinjection) and repeating at one or two hour intervals, attempt to strip ova from each female. Fertilize and start incubation of each batch of eggs. Continue until ripe eggs no longer produced, or until lack of response evident.

third day: fertilized eggs in incubation

fourth day: early morning, yolk sac larvae available to initiate feeding to skipjack larvae