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A summary of the symposium on rockfishes and recommendations for future research

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Significant progress in our understanding of the reproduction and aspects of the early life history of *Sebastes* was evident in the papers presented at this symposium (not all published in this volume). On the final day, we presented short summaries of this progress made in the various research topics – reproduction, embryonic and larval development, early life history, ecology, mariculture and fisheries applications – and considered the questions and discussions raised during the symposium for further research. A discussion session followed. This paper presents the summaries and, to the extent possible, incorporates points made during the discussion session.

Reproduction

The papers on reproductive biology encompassed several levels of biological organization, including biochemical endocrinology, cellular aspects, development and behavioral ecology of reproduction. New scientific approaches and techniques and integration of established techniques combined to demonstrate common patterns in rockfishes as well as species-specific differences among the different levels of organization. The paper of Akihiko Shinomiya elegantly described, for the first time, courtship and copulatory behavior in *S. inermis* and *Sebastiscus marmoratus* and included underwater video recordings. Courtship and copulation occur at dusk, but mating behavior and seasonality differ between the two genera. During courtship, males exhibit territorial behavior not evident in other seasons; females copulate with more than one male. Future research should extend these studies to other species, especially those occupying different habitats (e.g., pelagic and deepwater species). The possible role of pheromones in reproduction should be investigated. In view of the taxonomic significance of the extrinsic swim bladder muscles, it would be interesting to determine whether they are used to produce unique, species-specific sounds during courtship and mating.

Hiroya Takahashi described histological methods used to analyze the stages in the male and female reproductive cycles of *Sebastes taczanowskii*. Comparison of the reproductive cycles in five other species of *Sebastes* suggests that gametogenesis in both sexes is prolonged in northern species and shortened in southern species. There are several obvious areas for future inquiry, including the physiology of sperm storage, competition from sperm derived from different males and the timing and mechanism of egg fertilization. Papers on endocrinological studies of the reproductive cycle followed. Yoshitaka Nagahama described changes in steroid hormone levels during the reproductive cycles of female *S. taczanowskii* and *S. schlegeli*, giving the first comprehensive description of the

endocrinology of the female reproductive cycle in viviparous teleosts. Estradiol-17β is the most important steroid involved in vitellogenesis, and 17α, 20β-dihydroxy-4-pregnen-3-one plays an important role in final oocyte maturation and the maintenance of gestation, but progesterone apparently does not play a significant role. Kazunori Takano described steroid hormone levels in female *Sebastiscus marmoratus*, a rockfish with multiple broods. Changes in hormone levels could be correlated with histological changes in the gonadal cycle, and the roles played by the hormones are similar to those in *Sebastes*. The two studies indicate that there is a commonality in the hormonal control of reproduction in viviparous rockfishes.

Akihiro Takemura described research on female-specific serum proteins (FSSPs) in *S. taczanowskii* using immunochemical procedures. The three FSSPs detected (two of which were categorized as vitellogenin) were utilized during oogenesis. Since the vitellogenin level was low during gestation, two of the FSSPs did not serve as a supplemental source of nutrients for developing embryos. The third FSSP was a major constituent of the ovarian fluid but not of the vitellogenic oocytes, and may play a role in gestation. Comparative studies of seven rockfishes in the genus *Sebastes* and *Sebastiscus* revealed the ubiquity of vitellogenin, whereas the third FSSP was absent in several species of *Sebastes*.

A series of six papers was then presented on the physiological ecology of reproduction and reproductive physiology and development. Ralph Larson discussed the relationship between seasonal cycles of reproduction and of deposition and utilization of metabolic reserves. Compared to most other ectothermic vertebrates, *Sebastes* seems anomalous because some of its species deposit reserves during the period of gametogenesis. Reserve deposition is evident in liver weight and as fat storage in the mesenteries. Reserves decline during winter, coinciding with the main period of reproductive activity – a result suggesting reserves are used in gametogenesis and also for survival during winter when food availability is low. A comparison of northern and southern species reveals interspecific difference in reserves that may be related to geographical differences in the seasonal abundance of food. Further research on this topic should consider timing of the reproductive cycle in terms of the availability of food both for the reproductive adults and the newborn young.

Tina Wyllie Echeverria discussed sexual dimorphism and its causal factors among 34 species of rockfishes. The level of dimorphism varied among species and species groups. Semipelagic species are generally more dimorphic than benthic species in size as well as morphometric measurements. These dimorphisms may reflect compensation in feeding ability in response to reduced size (i.e., larger pectoral fins and eyes could aid males in competing for food) and may be related to mating behavior (larger fin size and head parts). Research to understand the causal basis of sexual dimorphisms would be fruitful. Differences between the sexes in the energetic requirements of reproduction may be related to the dimorphisms.

George Boehlert described research on the physiological energy costs of livebearing to the gravid female as measured by oxygen consumption of the females and the developing embryos. The major cost of livebearing to the gravid female is the energy expended to maintain the environment of the developing embryos, and the metabolic costs apparently are high. It was suggested that the gravid females respond to the increased energetic demands of gestation and reduce their overall metabolism either by decreasing feeding or by seeking lower temperatures. Both hypotheses can and should be tested. The temperature hypothesis is particularly intriguing because it suggests that viviparity with very large brood size is impractical in tropical and subtropical waters, and hence could explain the absence of *Sebastes* from those geographical regions.

Maxwell Eldridge characterized the annual reproductive cycle of the yellowtail rockfish, *S. flavidus*, at different levels of organization, ranging from the community-population level to the subcellular level. The reproductive profile of a southern population differed significantly from that of northern populations. The former population exhibited an annual reversal in sex ratio not previously observed in rockfish. Although the gonadoso-

matic index accurately profiled the seasonal reproductive cycle for both sexes and proved useful in separating immature from mature fish, it was not as sensitive as histological analysis of the gonads. The experimental program used in this paper provides a comprehensive method for comparing the reproductive profiles of different populations of the same species and the profiles of different species.

Lewis Haldorson reviewed published reports for data describing fecundity, maturity, growth and weight in 54 species in the genus *Sebastes*. Information on maturity and fecundity was examined for patterns associated with taxonomic relationships; this approach allows analysis of rockfish reproduction in the context of life history models. Rockfishes display a great range in size at maturity (9–52 cm), and maximum fecundity can attain a value of 5.60×10^6. Comparisons of exploited and nonexploited populations of rockfish may provide the means for testing various life history models, as elaborated in the later presentation by Bruce Leaman.

John Wourms reviewed viviparity in fishes, gave an overview of selected aspects of rockfish reproduction and development and speculated on the evolutionary origin of viviparity in rockfishes. Among the eight subfamilies of the Scorpaenidae, viviparity is confined to the subfamily Sebastinae; gestation is lumenal and the embryos usually develop to term within the egg envelope. Transitional states from oviparity to viviparity are evident in different species within the family. Although viviparity is best developed in the genus *Sebastes*, it is still primitive and unspecialized. Rockfish viviparity is essentially lecithotrophic (i.e., embryonic nutrition is dependent on the energy reserves laid down during oogenesis). In other groups of viviparous fishes, lecithotrophy has been shown to be better suited energetically to seasonally unpredictable habitats, whereas matrotrophy requires a predictable food supply. During the evolution of viviparity in rockfishes, the advantages of high fecundity associated with oviparity were retained while an enormous increase in the survival rate of the developing embryos was acquired. The basic lecithotrophic pattern of oviparous development did not change since it offered selective advantages both in terms of energetics and as a basis for retaining a large brood size. Comparative studies on reproduction and development of the less well-known sebastine genera *Hozukius* and *Helicolenus* would be beneficial to our understanding of the evolution of viviparity in this group.

Functional aspects of embryos and larvae

Against the rather comprehensive background of reproduction in adults presented in the first section, the second section dealt with various aspects of embryonic and larval development. The papers ranged from descriptive to experimental and demonstrated how the integration of reproductive functions of the female serves to promote internal development of fertilized eggs and embryos through to parturition. The lack of an accurate description of development of *Sebastes* embryos has impeded research comparing development in different species. Livebearing precludes continual observation of the normal developmental events in an individual embryo and prolonged in vitro observation may result in changes from the in vivo pattern (Triplett 1960). Past researchers have either used a modification of commonly used developmental schemes for oviparous fishes or, in at least one case, have developed a new, less detailed scheme specific to *Sebastes* (Stahl-Johnson 1984). Juro Yamada described development in *S. schlegeli* using the former approach. This staging system should serve to improve communication among researchers in *Sebastes* intent on relating results among different species and different laboratories. It can also serve as a point of departure for future studies on the structural aspects of development in *Sebastes*; highly detailed descriptions of structural development of *Sebastes* embryos would be useful to relate to the functional features described for embryos in several papers. Comparative studies with development of *Hozukius*, *Helicolenus*, and *Sebastiscus* could also aid in understanding the evolution of viviparity within the Scorpaenidae.

Yamada also described a technique refining the methodology for determining gestation period, using a great deal of data from *S. schlegeli* from a

mariculture laboratory in Japan. This approach appears to be a marked improvement over earlier estimation techniques and produced different results. Boehlert et al. (1986) suggested that the gestation period for this species was 51.5 days, whereas the new technique provides an estimate some 8 days shorter. It is interesting to note that the revised estimate would decrease the catabolic energy consumed, a concern noted in the paper by Boehlert, Kusakari and Yamada and also in the presentation by Boehlert on embryonic energetics.

Frank Conte outlined the ontogeny of the 'sodium pump' in developing embryos and demonstrated their ability to osmoregulate. The titer of the enzyme in embryos of both *S. schlegeli* and *S. taczanowskii* indicated that significant enzyme activity was not present until near stage 18, in general agreement with the results of incubations with saline containing the inhibitor ouabain. One might question the need for osmoregulation by embryos in the benign ovarian environment, since presumably the female can osmoregulate for the embryos and embryos are unable to osmoregulate for long in vitro. In the ensuing discussion, Teruyuki Nakanishi noted that rearing efforts for several species of marine fish larvae in Japan have benefited from using diluted seawater. Rearing trials at salinities 60–70% that of full strength seawater resulted in significantly higher survival rates than in full strength seawater. It is possible that the energetic costs of osmotic and ionic regulation may be put to use for other metabolic purposes during this period of rapid growth.

Boehlert described past work on embryonic energetics, comparing the results from species studied to date. The presentation and ensuing discussion raised several questions. There was some concern over the true rate of respiration of embryos; Conte suggested that incubation with curare in vitro would minimize the muscular activity in the later stages to provide estimates of resting metabolic rate. The question of oxygen tension in the ovarian fluid and the rate of passage through the larger ovaries of some species is also pertinent here. Given that developmental rates are very slow compared to oviparous fishes with similar egg sizes, the control of developmental rates of embryos would be interesting to study. Possible candidates include oxygen delivery by the female system or some level of hormonal control. A simple experiment was suggested in which embryos would be incubated in physiological saline in vitro and the developmental rate at different stages compared with in vivo rates. This has been done with embiotocid embryos by Triplett (1960), who observed earlier loss of spatulate fins and metamorphosis in vitro. He raised the question of potential hormonal involvement in the maternal-fetal relationship. Possible endocrinological links or other chemical communication is unknown in *Sebastes*, but the approach of Nagahama, Takano and their coworkers may answer many questions. Such a study could also address the question of a signal that embryos are ready for birth, if one exists. Finally, this paper pointed out the need for studies on pre- and postfertilization fecundity: are there atretic eggs from unfertilized ova or simply such are the result of embryo death? Eldridge's paper suggested no difference between pre- and postfertilization fecundity in *S. flavidus*, whereas Kusakari documented such a change in *S. schlegeli*.

The two papers on nutritional uptake by embryos took different approaches but reached similar conclusions. The radiochemical approach described by Mary Yoklavich suggests uptake of exogenous substrata at all stages examined, but utilization only in late stage embryos after the point of gut development. Results from autoradiography unfortunately were not consistent. Although a site of uptake could not be traced to the ultrastructural level, histological examination did suggest some epidermal uptake in an early embryo. This was confirmed in several embryo stages in the paper presented by Motohiro Shimizu, who used ruthenium red to demonstrate uptake in the epidermis. Given that the radiochemical results with an amino acid do not support utilization of exogenous substances in the early stages, one might question whether this uptake is nutritionally meaningful. The hindgut is clearly the site of pinocytotic uptake of substances. After the mouth opens, ingestion of ovarian fluid results in uptake in the hindgut, which is morphologically similar to that in other feeding fish larvae. Further research will be necessary to

identify the source of the exogenous substances consumed: are they intraovarian or supplied from the maternal system? The approach of Takemura and his colleagues could be supplemented by a radiochemical study with peptides or proteins of different molecular weight to address this question. The final histological question identified as needing more research was on the function of sacciform cells. As demonstrated by Shimizu, the sacciform cells are highly abundant, but their function remains unknown (Bullock 1980). They appear, however, not to be absorptive.

Nakanishi described work on the development of the immune system in *Sebastiscus marmoratus* larvae. Again, the issues of the generic identity and the relationship of *Sebastiscus* and *Sebastes* were raised, making comparative work with the latter genus of interest. Nakanishi's approach provides encouragement for further research on the development of organ systems not only in larvae but in embryos as well. The late elaboration of the lymphoid system in *Sebastiscus marmoratus* again raises the question of hormonal control of organogenesis in embryos. In the case of the lymphoid system, however, it is not under maternal control, because development does not occur until well after parturition.

Identification, systematics and ecology

The presentations by Arthur Kendall and Lisa Seeb showed that the last few years have seen major advances in the identification of *Sebastes* larvae and juveniles. These advances have come from two very different directions. One is the traditional methodology utilizing superficial morphological characters. While a number of *Sebastes* larvae may now be identified through examination of cranial spines, pigment spots and other characteristics, the limits to the utility of this approach may be near. It is quite possible that a number of closely related species may not be distinguishable using this protocol. Seeb's presentation takes us to the next step, the use of molecular techniques such as allozyme analysis and mitochondrial (mtDNA) and nuclear DNA restriction fragment polymorphism analyses. However, the usefulness of these more esoteric techniques will depend on several factors. Perhaps the most important is a data base developed for adult *Sebastes* that will allow identification of larvae and juveniles. Second, the laboratory techniques needed for these methods have not yet been fully codified.

Research on *Sebastes* systematics, an area given little attention in recent years, is approached in several imaginative ways. Kendall shows that rockfish larvae and juveniles may be characterized using various morphological characters. These species may also be grouped by these characters, and these groups compared with currently accepted subgeneric alignments. This technique met with only limited success, indicating that some subgenera are probably artificial species groups with convergent morphologies.

In reference to Seeb and Kendall's paper, DNA techniques for research on *Sebastes* systematics point the way to the future in this field. Indeed, the great sensitivity of various DNA techniques (such as mtDNA and nuclear DNA restriction fragment polymorphisms) may prove to be a two-edged sword. For instance, Seeb mentioned the difficulties in distinguishing the putative sibling species *S. carnatus* and *S. chrysomelas,* even with mtDNA analysis, thereby implying greater complexity in rockfish systematics. The application of DNA techniques to systematics research may therefore force the use of other methods, such as crossbreeding, to sort out relationships.

Geoffrey Moser presented a wealth of information on distribution and seasonality of *Sebastes* larvae off California and Baja California. His paper is a badly needed synthesis of an important time series of data and will help us in understanding the distribution patterns of larvae. Still needed, however, are further explanations for some of these patterns, particularly those environmental factors that contribute to annual variation in larval incidence and abundance. Better taxonomic resolution will be necessary to provide much of this analysis.

Moser also discussed the pelagic juvenile interval of *Sebastes*, summarizing work that represents a major advance in our understanding of an area for

which relatively little data were previously available. However, as with the larval stages, a number of questions remain. Among these are the factors leading to enhanced juvenile survival and how these young fish find suitable habitat for recruitment. Indeed, the question of rockfish navigation remains something of a mystery.

Milton Love focused on juvenile *Sebastes* ecology. Recent research has given us a much better idea of the recruitment patterns of shallow-water *Sebastes*. New data are available on annual variability in inter- and intraspecific recruitment magnitude. Recruitment variation now appears to be strongly influenced by pelagic juvenile availability. Algae and other attached macrophytes play an important role in the recruitment process. However, whether these plants are essential for these recruits is not known. Algal removal experiments would be a natural follow up.

Various types of movements have recently been examined. Of particular interest are the ontogenetic shifts of newly recruited juveniles from *Macrocystis* and *Nereocystis* canopies down to understory benthic algae. Considerable data indicate that seasonal movements occur. Storm-derived turbulence, in particular, may drive young-of-the-year rockfish from nearshore habitats, though their destination is unclear. Increasing attention has been focused on diel movements, and indications are that these movements are complex, with activity patterns changing as the young-of-the-year mature.

The role of the large schools of juvenile rockfish in kelp beds in regulating invertebrate populations through predation on larvae has been given some attention, but pertinent experiments have not been done. For instance, it would be a simple matter to measure plankton entering and exiting a kelp bed during daylight hours when rockfish are most actively feeding, and at night when predation is reduced.

Lastly, virtually no information is available on the ecology of deepwater juvenile *Sebastes*. It is hoped that directed research with remotely operated vehicles and manned submersibles will make greater advances in this area.

Mariculture and fisheries

In order to enhance depleted stocks of *Sebastes*, three major aspects must be considered. First, improvement of mariculture technology is necessary to raise large numbers of juveniles as efficiently as possible. Second, ecological evaluation of the effects of releases of cultured juveniles on stocking and fishery yield must be considered. Third, in connection with the second, is the justification and selection of target species based upon life history and economic grounds.

The current status of technology for mariculture of *S. schlegeli* as developed in Hokkaido, Japan, was presented by Kusakari, who showed that rearing of larvae up to 10 cm juveniles can be attained with relatively low mortality on the order of 45%. Major problems in this process are diseases causing mortality. Studies of immune systems during ontogeny, as described in *Sebastiscus marmoratus* by Nakanishi, will be needed for more effective culture of juveniles. The possibility of endocrinological control of reproduction should be examined for improvement of brood stock management and collection of larvae. In this connection, it is important to identify the hormones involved in the maturation of gonads, mating behavior, internal fertilization, maintenance of gestation and parturition. This approach should be encouraged by the results presented by Nagahama and Takano. Seasonal cycles of reproduction in various species were addressed by several authors; changes of energy reserves by Larson, comprehensive analysis of reproductive performance by Eldridge, female-specific serum proteins by Takemura, gonad maturation by Takahashi and Takano, all are important factors for brood stock management. Developmental stage analysis and the time course of embryonic development, beyond that tentatively presented by Yamada, are also essential knowledge for aquaculture.

The theoretical effects of releasing hatchery raised juveniles on stock of *Sebastes alutus* were described by Jeffrey Polovina. Based on a simulation using the Deriso-Schnute model, he proposed that hatchery releases can potentially restore

a depleted population and may be economically feasible if juveniles are raised at a cost below US $0.15 per individual. Kusakari commented that the present cost for raising one 10 cm juvenile *S. schlegeli* is 70 yen (equivalent to US $0.50), but this cost can be reduced if the economics of scale are achieved as in the case of *Paralichthys olivaceus* now being produced on a large scale in Japan. Culture of larvae to juveniles of an optimal size implies lowering the natural mortality in the early life stages by protecting them from predation with a sufficient supply of prey. Release of cultured juveniles, therefore, can cause changes in the ecological traits of a population through a density change. Leaman discussed compensatory responses to exploitation of *Sebastes* populations based on life history theory as applied to *S. alutus*. Impacts of increased recruitment through stocking can be analyzed on the same basis, but as the reverse of exploitation. Long-term analyses of tagging and recapture experiments of released fish are necessary.

In Japan, the mariculture project started without much knowledge of life history and ecology of each target species. Some coastal species of *Sebastes* and *Sebastiscus* were considered to be best suited to mariculture because of their peculiar mode of reproduction, high fecundity and presumed nonmigratory behavior. These criteria should be reexamined in light of Leaman's analyses, in which *Sebastes* species were characterized as iteroparous long-lived fishes.

In the ensuing discussion session, one of the most lively topics was why North Americans have been unable to rear *Sebastes* with the success of the Japanese. Mortality rates in studies in the United States have been extremely high compared to Japanese efforts, with few, if any, juveniles reaching ages in excess of 50 days (Fig. 1). Several reasons were offered, including the smaller larvae in the eastern Pacific, the lack of sophisticated food rearing capabilities and the frequent use of embryos that were artificially extruded. On the latter point, the importance of using embryos born naturally to females after carrying them through the full term of gestation was noted. It was concluded that there is

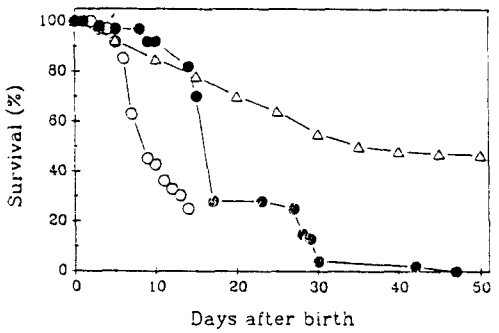

Fig. 1. Survival rates of *Sebastes* larvae in rearing trials, comparing efforts in the United States and Japan. Triangles, *S. schlegeli* (Kusakari this volume); filled circles, *S. melanops* (Boehlert & Yoklavich unpublished data); open circles, *S. auriculatus* (Stahl-Johnson 1984).

no a priori biological reason for this problem, but rather that it is a technological one; rearing attempts in the United States have often been on an ad hoc basis and not with funding dedicated for the purpose. Although the larvae are significantly smaller, this has not impeded complete rearing of larvae of other fishes.

Considerable discussion also occurred on the importance of further research and the factors impeding research progress. Availability of funding was a real concern. It was recommended that U.S. investigators and the agencies that support their work incorporate the experimental, laboratory-based approach into the investigation of the biology of fishes. There is need for basic research in morphology, physiology, genetics, life history, development and cell biology both in rockfishes and fishes in general.

For many reasons, the study of fish reproduction and the development of viviparous fishes such as rockfishes is both important and interesting. First, fishes are the most abundant, most diverse and yet the least known group of vertebrates. Second, fishes are the most primitive vertebrates and include the lineage from which tetrapods, including mammals and man, evolved. Accordingly, they are ex-

cellent models for elucidating the origins of vertebrate developmental mechanisms. Third, as a diverse group, fishes exhibit a diversity of developmental patterns, so that it is possible to find a 'system' that will be applicable to many problems. Fourth, our current knowledge of vertebrate development is actually very poor, because it is based on a very small and biased sample. Finally, fishes as a group are of major economic importance. With the decline in populations of wild stock and the increase in number of species used in aquaculture and mariculture, intensive study of fish reproduction and development becomes imperative if these enterprises are to succeed. Many of the same arguments used to justify the study of fish development in general can be extended to the development of viviparous fishes including rockfishes. Several additional features should also be considered. Viviparity makes its first appearance in the vertebrates among fishes. Viviparity has repeatedly and independently evolved from oviparity among fishes. The evolution of vertebrate viviparity led to the establishment of specialized maternal-fetal relationships. Nearly all of the adaptations for viviparity that occur in higher vertebrates, including mammals, first appear in fishes. Fishes also possess unique adaptations.

Epilogue

The papers presented at this symposium have increased the level of our knowledge on the reproduction and early life history in the genus *Sebastes* and have developed new techniques and approaches. As is characteristic of good research on new problems, however, the papers raise more questions than they answer. We hope that future collaboration and cooperation will be fostered by this meeting and the publication of the proceedings and will result in an effort to develop research to address the questions.

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