IDRC-TS21e

The Theory and Practice of Induced Breeding in Fish

Brian J. Harvey and William S. Hoar

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Theory and practice of induced breeding in fish. Ottawa, Ont., IDRC, 1979. 48p. : ill.

/IDRC publication/, /fish culture/, /fish breeding/, /tropical zone/ — /reproduction/, /fertilization/, /hormones/, /artificial insemination/, /theory/, /methodology/, /literature survey/.

UDC: 639.3.034.2 ISBN: 0-88936-236-X

Microfiche edition available

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THE THEORY AND PRACTICE OF INDUCED BREEDING IN FISH

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ACKNOWLEDGMENTS

Sincere thanks are extended to E.M. Donaldson for reading the manuscript critically and making constructive suggestions. We are also grateful to the following who commented helpfully on sections relevant to their areas of interest or research: W.H.L. Allsopp, T.J. Lam, B. Morley, N.E. Stacey, W.E. Vanstone, and F.C. Withler. Illustrations were prepared by F. Zittin, with the exception of Fig. 6, which was prepared by B. Bysouth. The carp logos, which introduce and end each chapter, were also designed by B. Bysouth.

FOREWORD

For many centuries fish farming has been a tradition among the Chinese and other rural peoples in Asia and elsewhere. Varro and Pliny describe the fish farms of late republican and early imperial Rome, with their shellfish beds off the coast of Latium and Campaina, and the fresh- and saltwater ponds stocked by the wealthy Romans with carp, mullet, tinbot, and eels. Varro, writing before the battle of Actium, describes how one Roman, Hirrius, made an annual income of 12 million sesterces from his fish nurseries.

Though fish farming has continued in Asia, fish cultivation as a commercial venture disappeared in Europe until the mid 19th century when the Frenchman, Coste, the father of the oyster industry, adapted the techniques of oyster culture he observed in the bay of Taranto, Italy, to establish his oyster cultivation industry in the Bay of Saint-Brieuc.

In spite of the long history of fish farming, only comparatively recently has the commercial cultivation of fin fish become established in Europe and North America. Now, the industrial production of Pacific salmon depends in large degree upon the proliferation of the young in the hatcheries of the Pacific Northwest.

Aquaculture, the name given to the systematic cultivation of living things in water, is primarily devoted to fish culture and is exciting interest in many countries; among fin fish, greatest attention has centred upon various species of carp and tilapia.

People's preference for and acceptance of fish, as of any other food, is largely conditioned by their environment and past experience. Ideally, therefore, one would seek to propagate and cultivate indigenous species that are familiar to and well accepted by local people. Unfortunately, many fish species do not readily reproduce themselves — the gravid females will not release their eggs in captivity. An adequate supply of fertilized eggs to provide the fry and juveniles is essential to every fish culture enterprise.

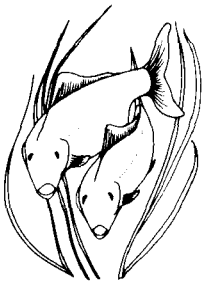
In 1934, in Brazil, von Ihering et al. discovered that reluctant female fish in captivity could be induced to breed if injected with extracts of fish pituitary glands containing gonadotropin sex hormones. Gonadotropin-induced breeding is now being widely studied and used in the breeding of carp in China and India, and of other species elsewhere. The Fisheries Research Board of Canada refined the technique for extracting the hormone, and scientists in the Philippines — supported by IDRC — applied the technique to milkfish (*Chanos chanos*), inducing them to breed by injections of Pacific salmon pituitary extracts. Refinement and wider adaptation of induced breeding of both indigenous and desirable exotic species, combined with improved systems of management, could help to realize the potential for the cultivation of many tropical species in many of the great rivers of the developing world including the Amazon, Brahmaputra, Congo, Indies, Mekong, Niger, and Nile.

As with animal husbandry, it will be necessary to breed and select superior species and strains according to their food habits, growth potential, disease

resistance, and acceptability. Combined with hormonally induced spawning, the cryogenic preservation of fish ova and milt (sperm) in liquid nitrogen offers remarkable opportunities for selective breeding and hybridization. More research is needed on techniques for incubation, larval rearing, and nutritional needs of juveniles and adults, and the efficiency of feed conversion at all stages of growth.

We in IDRC believe that a concise compilation of the state of theoretical and practical knowledge is urgently needed in the field of induced breeding and that Drs Harvey and Hoar are eminently qualified to provide it. I am confident that this publication, based on a literature survey completed 30 July 1979, will prove of great value to all who are engaged or interested in the science and practice of aquaculture.

Joseph H. Hulse
DIRECTOR
AGRICULTURE, FOOD AND
NUTRITION SCIENCES



1. INTRODUCTION

The successful large-scale cultivation of any organism for human consumption demands that the resource be easily renewable. Removal of the standing crop in agriculture, for instance, is followed by reseeding, and the reproduction of cattle can be assured either through natural means or by artificial insemination. It is clearly disadvantageous to cultivate any organism when the supply of young cannot easily be replenished; yet the history of aquaculture has been dogged by just this problem. Of the numerous species of fish under cultivation throughout the world, few — a notable example is *Tilapia* sp. — will breed in captivity (Jhingran and Gopalakrishnan 1974). In those species that will, for example the common carp *Cyprinus carpio*, the time of spawning is not entirely predictable and survival of fry under natural conditions may be unacceptably low. There are two solutions to this problem: the first is eminently practical and has been the historical answer — namely to collect fry from natural sources. Yet the difficulties inherent in this method are many, for it is time consuming, requires considerable skill and experience, and relies utterly on the productivity of the natural spawning grounds. The second solution is more direct: learn how to make the fish reproduce in captivity. It is this approach that has received the most attention in recent years and has borne considerable fruit.

Reproductive processes are by no means fully impaired in captivity. The progressive development of the gonads remains, in general, uninhibited up to the final stages of gamete maturation, and it is only at the point of gamete release that the sequence is arrested. Both gonadal maturation and spawning behaviour have long been known to be responses to environmental stimuli — temperature, hours of daylight (photoperiod), and the amount of rainfall among them — and the fish culturist is fortunate in that it has proved possible to intervene successfully at the stage where the needed environmental cues are lacking and to push the process to an artificial completion. This has been accomplished through the technique of induced breeding through hormonal manipulation, and it is the practical application of this technique that is the main subject of this review.

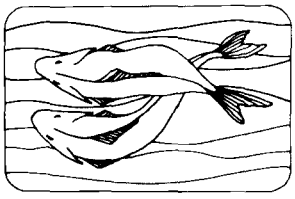
Though the primacy of environmental stimuli in teleost reproduction should not be lost sight of and though there exists a vigorous research effort directed toward a fuller understanding of these influences, hormonal induction represents a practical solution that by "forcing the issue" tends largely to ignore them. The control of reproduction solely through the manipulation of environmental conditions is not, therefore, considered here, nor is the endocrinology of sexual behaviour, for this, too, is denied a role whenever complete control of gamete release and fertilization is desired. It is assumed throughout this review that, from the standpoint of the fish culturist, the desired end in a program of induced breeding is the production of viable gametes, which may then be used to effect fertilization under controlled conditions. Natural fertilization can only occur when spawning behaviour is fully expressed; it is time consuming, requires considerable space, and involves attendant losses of fry. It is, therefore, not regarded as practical.

It is recognized that the audience for the following review will include readers of widely differing backgrounds. An interest in fish breeding may, after all, be common to a university-based fisheries biologist and a fish farmer in rural India, although their concerns are likely to be different.

The purpose of this review is, therefore, twofold: first, to summarize and discuss recent advances in the knowledge of the reproductive physiology of fishes that affect our ability to spawn cultured species in captivity; and second, to extract from the literature the practical details of methods of induced breeding currently in use and to present them in such a way that consistencies in technique are highlighted. Accordingly, an attempt has been made to segregate the theoretical material from the practical. Field-oriented readers, whose main interest lies with the reproduction of their own broodstock, may thus turn directly to chapter 4, a survey of recent published attempts to induce breeding in the following species: carp (common, Chinese, and Indian major carps), catfish (Asian varieties), mullet, and milkfish. Information concerning carp and catfish is presented in tabular form, and these tables may be consulted as a source of ideas for culturists

wishing to benefit from the experience of others in their field. Chapters 2 and 3, on the other hand, present the theoretical underpinning for the practical methods and will be of most interest to the reader possessing a solid background in basic biology. A short account of teleost reproductive endocrinology is presented in chapter 2, whereas chapter 3 deals with recent advances in the hormonal manipulation of reproduction. Chapters 5 (Methods of Ovarian Biopsy) and 6 (Preservation of Gametes) will be of interest to researchers and culturists alike. Suppliers of spawning materials, including hormones and whole pituitary glands, are appended.

The injection of crude piscine pituitary extract to induce ovulation is referred to as "hypophysation," and was first successfully demonstrated by von Ihering and coworkers in Brazil in 1934 (von Ihering 1937). This technique, although still forming the backbone of many induced breeding operations, has over the intervening years generated increasingly sophisticated solutions to the problem of reproductive refractoriness, to the point where purified gonadotropins, hypothalamic releasing hormones, hormones of mammalian origin, sex steroids, and such "extrabiologic" substances as the antiestrogen clomiphene, have all been employed with various degrees of success. There exist numerous recent reviews of the subject (see, for example, Chaudhuri 1976; Fontaine 1976; Shehadeh 1972 and 1976), addressing topics such as preparation and storage of pituitary extracts, phylogenetic specificity of administered hormones, selection of breeders and the assessment of their state of gonadal development, standardization of dosage, and the preservation of sex products. The emphasis in the present review will be on fish cultivated in tropical and subtropical countries, although significant developments in temperate regions, such as the recent use of releasing hormones in spawning carp in China, will be introduced.



2. TELEOST REPRODUCTIVE ENDOCRINOLOGY

The nervous and endocrine systems of vertebrates act in concert to coordinate reproductive events. Major links in the chain of events leading from the perception of environmental stimuli to the release of gametes are depicted diagrammatically in Fig. 1, and it can be seen from this diagram that neural events predominate in the early stages, whereas later links in the chain are hormonal.

The reception of such environmental stimuli as daylength (photoperiod), temperature, and the amount of rainfall is mediated by the nervous system and involves the passage of information from sensory receptors to the brain. This neural information, upon reaching the hypothalamus, determines the activity of the pituitary gland by way of chemical messengers termed releasing hormones. These, in turn, stimulate the pituitary to release into the general circulation a hormone whose target organ is the gonad. This hormone is termed a gonadotropin. Its effect is to stimulate the production of sex steroids on the gonad; these, then, are responsible for the maturation of gametes. The transition from neural information to hormonal control takes place at the interface between hypothalamus and pituitary, and it is here that our detailed consideration of the endocrinology of reproduction in fish begins.

PITUITARY AND HYPOTHALAMUS

The embryonic origin of the pituitary gland of vertebrates is twofold. An epithelial component, derived from the embryonic mouth cavity, is termed the adenohypophysis. It produces, in addition to the gonadotropic hormone with which we are largely concerned, somatotropin (growth hormone), corticotropin, prolactin, thyroid-stimulating hormone, and melanocyte-stimulating hormone. The adenohypophysis may be subdivided anatomically into rostral pars distalis, proximal pars distalis, and pars intermedia (Fig. 2). A neurally derived component, the neurohypophysis, connects the adenohypophysis to the base of the brain and is composed largely of the axonal fibres of neurons whose cell bodies are located in the hypothalamus. This nervous tissue interdigitates extensively with the adenohypophysis, particularly in the pars intermedia, and the presence of this nervous "core" has given rise to the concept of the pituitary as a "dual" organ.

Hypothalamic neurons whose axons make up the neurohypophysis are of a specialized type referred to as neurosecretory cells. These respond to an electric signal from the brain with the release of a chemical messenger at the axon terminal, thus bridging the gap between nervous and hormonal information. Their cell bodies form several distinct groups or "nuclei" within the hypothalamus, which may be distinguished both anatomically and on the basis of their staining properties. In the teleost pituitary the two most important for our purposes are the nucleus preopticus (NPO) and the nucleus lateralis tuberis (NLT). Hormones produced by NPO neurons are released largely into a blood channel running between neurohypophysis and adenohypophysis; some may innervate directly the cells of the pars intermedia (Ball and Baker 1969). Our main interest, however, lies with the neurons of the NLT, and the arrangement of these is unique to teleosts. Endocrine cells of the adenohypophysis are here seen to be innervated *directly* by the neurosecretory axons (for the sake of simplicity only gonadotropic cells are shown in the figure), and the chemical messenger released at this junction is termed a releasing hormone (RH). The effect of the releasing hormone is to stimulate the production of gonadotropin and its subsequent release into the vascular system of the adenohypophysis. Gonadotropin is then carried by way of the systemic circulation to the target organ, the gonad, where it in turn initiates the production of the sex steroids. These hormones — androgens, estrogens, and progestogens — are the direct mediators of gonadal development.

Evidence for the hypothalamic control of gonadotropin release from the teleost pituitary is provided by experiments in which electrolytic lesions of the NLT of the goldfish *Carassius auratus* caused a decreased gonadosomatic index, blocked ovarian recrudescence, and induced follicular atresia (Peter and Crim 1978). The concept of a GRH centre in the hypothalamus is supported by the presence of

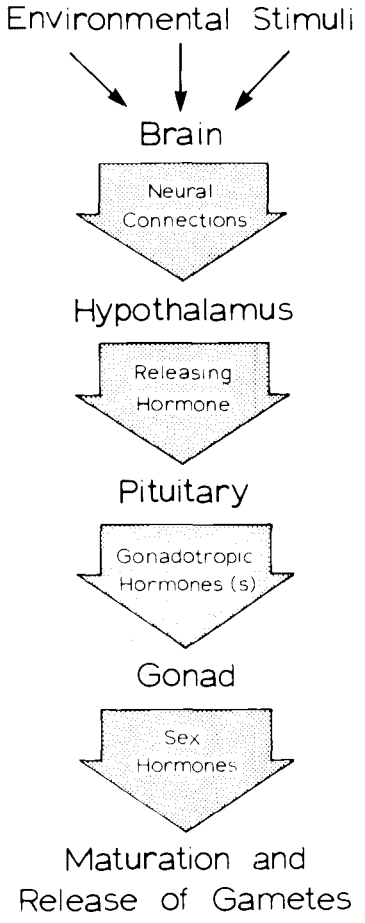


Fig. 1. *Major links in the physiologic chain of events leading from the reception of environmental stimuli to the release of mature gametes.*

GRH activity in extracts of this region in various species of teleost fish (Crim et al. 1976). The chemical nature of the releasing factor in teleosts remains unresolved. GRH activity in carp hypothalamic extract is associated with a substance whose molecular weight is less than 5000; the neurotransmitters epinephrine, norepinephrine, serotonin, and dopamine have no GRH activity on carp pituitaries in vitro (Breton et al. 1975b). The fact that releasing hormones are relatively small molecules is significant. It suggests the feasibility of synthesizing the hormone or an equally active analogue that may stimulate maturation of the gonad by increasing the production of gonadotropic hormone. Encouraging preliminary results have, in fact, been achieved using this approach (chapter 2).

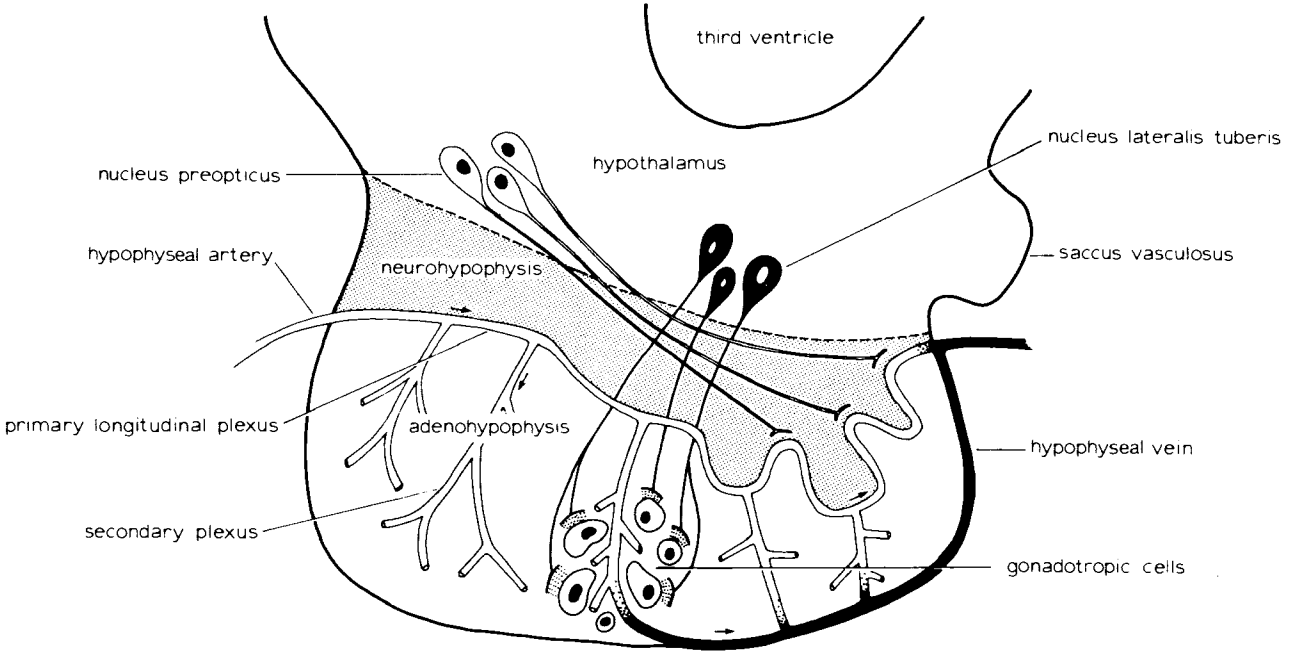
TELEOST GONADOTROPINS

Gonadotropins have been isolated in relatively pure form from several teleosts, including the carp *Cyprinus carpio* (Fontaine and Gerard 1963; Burzawa-Gerard 1971), chinook salmon *Oncorhynchus tshawytscha* (Donaldson et al. 1972), and *Tilapia mossambica* (Farmer and Papkoff 1977). There is considerable evidence that fish elaborate two distinct types of gonadotropic hormone. An ovulatory action is ascribed to only one of these, which is high in glycoprotein (Donaldson 1973; Farmer and Papkoff 1977). The other gonadotropin is low in glycoprotein and is believed involved only in the control of vitellogenesis (Idler et al. 1975; Ng and Idler 1978). The presence of two chemically distinct gonadotropins is indicated by cytologic and histochemical evidence (Burlakova and Belyanov 1976; Schreibman et al. 1973), and sexual differences in isolated gonadotropins have been reported (Breton et al. 1978). This confusing picture will be clarified by a systematic search for gonadotropic activity at different stages of gonadal maturation (Fontaine 1976).

Given the protein nature of gonadotropic hormones, it is not surprising that phylogenetic specificity has repeatedly been demonstrated. Although certain broad similarities may extend even across phyla — the *Tilapia* gonadotropin for example exhibits chromatographic identity with a variety of mammalian and nonmammalian luteinizing hormones (Farmer and Papkoff 1977) — there exist partial zoological specificities in teleost gonadotropins. Fontaine et al. (1972) have demonstrated that the activity of the gonadotropin of carp is 36 times that of *Oncorhynchus* when assayed in terms of adenyl cyclase activity in the goldfish, and it is a commonplace of hypophysation technique that the effectiveness of a homoplastic pituitary extract (from a fish of the same species) may well exceed that of a heteroplastic donor. There are exceptions to this — the common carp, for example, is widely used as donor for both Chinese and Indian major carps — but the problem has real significance for the culturist engaged in hypophysation and will be returned to in the course of this review.

MATURATION OF THE GONAD IN RESPONSE TO GONADOTROPIN

It is currently believed that maturation of the gonads in fish proceeds as an indirect result of a slow and steady rise in gonadotropin secretion and that ovulation and spermiation are preceded



Subdivision	Rostral Pars Distalis	Proximal Pars Distalis	Pars Intermedia
Hormone Produced	Corticotropin Prolactin	Thyrotropin Somatotropin Gonadotropin	Melanocyte Stimulating Hormone

Fig. 2. *Diagram illustrating the organization of the teleost pituitary gland, including the blood supply to the organ. Anterior to the left. Blood enters the gland from the hypophyseal artery, passes to the primary longitudinal plexus between neurohypophysis and adenohypophysis and from there to the adenohypophysis by way of the secondary plexus. It is then collected in a superficial venous network (black) and exits by way of the hypophyseal vein. Arrows indicate the direction of blood flow. A group of gonadotropic cells is shown in the adenohypophysis; these release gonadotropin into the secondary plexus upon stimulation by releasing hormones liberated from the axons of nerve cells of the nucleus lateralis tuberis. Dotted lines represent releasing hormone. Modified from Ball and Baker (1969) and Perks (1969).*

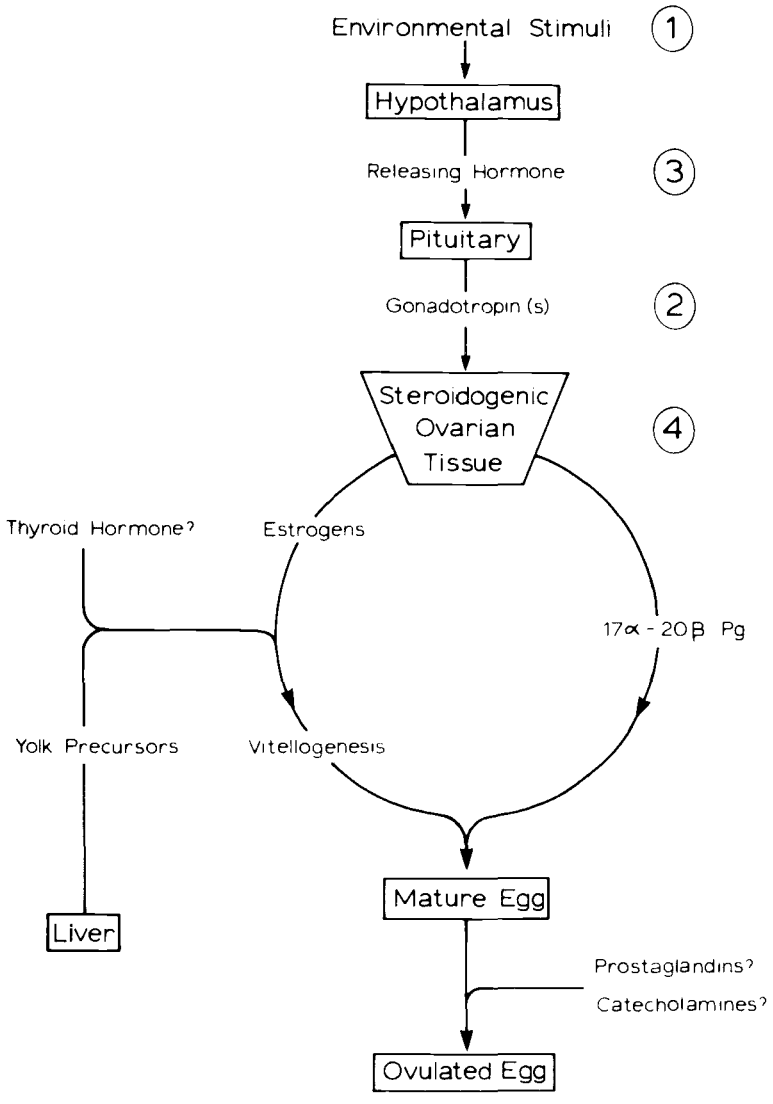


Fig. 3. *Endocrinological links in the chain between the reception of environmental stimuli and ovulation. Circled numbers refer to those stages where artificial intervention has been at least partially successful in bringing about ovulation in captive fish.*

by a more marked increase. This hypothesis is borne out by evidence from salmonids (Breton et al. 1975c; Crim et al. 1975) and cyprinids (Breton et al. 1972; Stacey et al. 1979). The action of gonadotropin in regulating the events of gametogenesis is largely indirect, by way of the steroid sex hormones, and this final link in the chain provides important clues to the possible causes for the interruption of maturation in captivity.

It is important to appreciate that gonadal maturation in males may very often *not* be interrupted in captivity and that there is usually little difficulty in obtaining milt from a pond-reared fish. Hypophysation of males, when it is carried out, is often a matter of convenience in ensuring precise coordination of gamete release and serves further to initiate a seminal thinning response that renders the milt more workable for purposes of artificial fertilization. For this reason the following discussion is confined to gonadal development in the female: oogonial proliferation, vitellogenesis, oocyte maturation, and ovulation

(Fig. 3). It is stressed that the process of spawning, or oviposition, is distinct from ovulation and may not be responsive to the same hormonal controls.

OOGONIAL PROLIFERATION

Oogonia arise from primordial sex cells in the germinal epithelium of the ovary and, at an early stage, become surrounded by a layer of epithelial cells termed the ovarian follicle. The early development of the ovarian follicle appears not to be pituitary dependent in fish. The cells of the follicular epithelium differentiate to form a glandular granulosa, separated from the ovum by a noncellular zona pellucida, and a distinct theca develops from the surrounding connective tissues. These structures — granulosa, zona pellucida, and theca — may be termed the follicular envelope (Fig. 4).

VITELLOGENESIS

Vitellogenesis involves the incorporation of yolk in developing oocytes and, as already noted, this process is believed to be under the control of a low-glycoprotein gonadotropin (Ng and Idler 1978). The target cells for pituitary gonadotropin appear to be the special thecal cells of the follicular envelope (Hoar and Nagahama 1978), and the sex steroids produced here play a major role in the regulation of this complex process.

Yolk is deposited in two forms, yolk vesicles and yolk granules, and their deposition is normally sequential. The formation of yolk vesicles, which occurs first, has recently been shown to be induced by estrogens in the goldfish (Khoo 1979); yolk granules are believed to be formed under the influence of pregnenolone. The synthesis of yolk precursors occurs in the liver and has been shown to be stimulated by estrogens (Ho and Vanstone 1961; Campbell and Idler 1976).

Thyroid hormones have also been implicated in vitellogenesis. Low doses of thyroxine stimulate vitellogenesis in immature goldfish (Hurlburt 1977a), and it is suggested that thyroid hormones act synergistically with gonadotropin to influence ovarian development, possibly by way of an increase in ovarian sensitivity to gonadotropin stimulation.

OOCYTE MATURATION

The final stages of oocyte and follicular development may be experimentally dissociated from ovulation (expulsion of the denuded oocyte from the follicle into the ovarian or peritoneal cavity). The steroid 17α-hydroxy-20β-dihydroprogesterone (17α-20β Pg), produced by the follicular envelope in response to pituitary gonadotropin, has been proposed as the most probable mediator of oocyte maturation in rainbow trout (*Salmo gairdneri*), northern pike (*Esox lucius*), and goldfish (*Caras-*

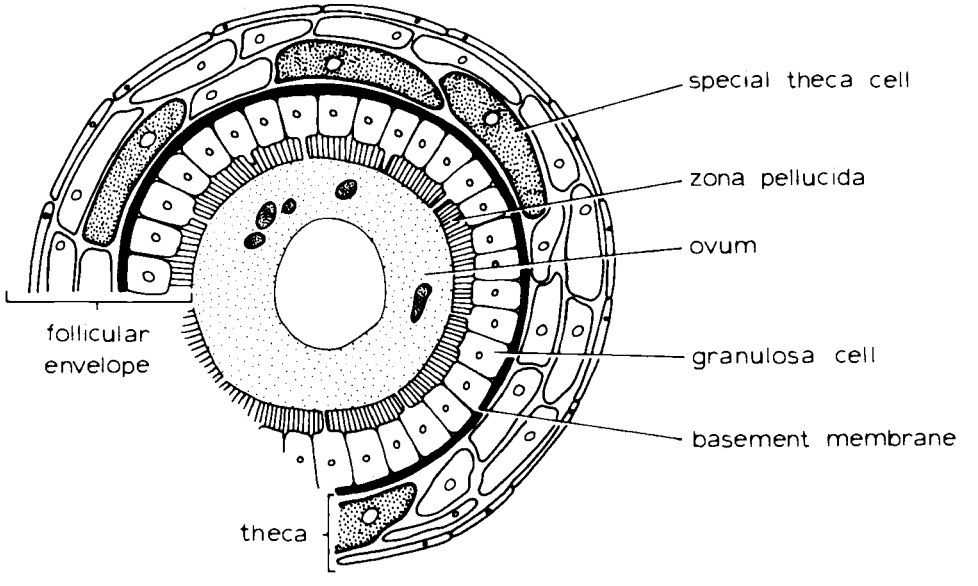


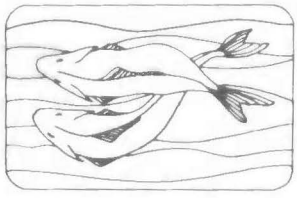
Fig. 4. *Diagrammatic representation of the developing ovum. Steroidogenesis stimulated by pituitary gonadotropin takes place in the special thecal cells of the follicular envelope. Redrawn from Hoar and Nagahama (1978).*

An intramuscular injection of pituitary extract is applied dorsally to a gravid female.

sius auratus) (Jalabert 1976). Corticosteroids may play an indirect role, possibly through sensitizing the oocytes to the action of 17α-20β Pg; these hormones assume a more significant role in oocyte maturation in catfish (Sundararaj and Vasal 1976; Jalabert 1976).

Ovulation

Follicular rupture and expulsion of the denuded oocyte appear to be independent of pituitary control. Both prostaglandins and catecholamines have been proposed as mediators (Jalabert 1976); in vivo evidence supporting a role for prostaglandins in goldfish ovulation has been provided by Stacey and Pandey (1975).



3. INDUCED BREEDING: THEORY

The aquaculturist carrying out a program of induced breeding is most concerned with stimulating ovulation. Oviposition (spawning) need not occur spontaneously; eggs can be stripped from the fish, and indeed this procedure is preferable if complete control of fertilization is to be achieved. To do this, however, the culturists must, first, ensure that final maturation, normally mediated by pituitary gonadotropin, is completed. At what stage or stages in the reproductive cycle can they intervene to achieve this? Fig. 3 indicates the stages in female maturation at which it has so far been possible to intervene to effect ovulation in captivity.

As has already been noted, manipulation of environmental parameters (stage 1 in the diagram), although the object of considerable research in temperate species, is beyond the scope of the present discussion. It should also be pointed out that current chemical intervention is directed toward that part of the sequence leading to final oocyte maturation only. Ovulation itself, though the ultimate goal of any stimulatory effort, appears to occur as the result of endogenous stimuli following the achievement of final oocyte maturation, although environmental factors may contribute. This leaves three stages (2, 3, and 4) listed in order of their relative importance in terms of practical successes. The administration of exogenous gonadotropin assumes by far the greatest significance; intervention at the hypothalamic–pituitary interface through the administration of releasing hormones (stage 3) shows considerable promise and has produced some recent practical results; intervention at the level of the sex steroids (stage 4), either through simple administration or through their feedback control, is at present the subject of experimental research.

ADMINISTRATION OF EXOGENOUS GONADOTROPINS (STAGE 2, FIG. 3)

Hypophysation refers to the injection, either intramuscularly or intraperitoneally, of crude extracts of piscine hypophysis or pituitary gland. The basic technique has been in existence for some time — preliminary experiments were performed by Houssay in 1931 — and has undergone considerable refinement over the years. It remains the only realistic choice for the rural fish farmer, for reasons both economic and technical. For information concerning methods of collection, processing, and storage of pituitary glands, the reader is directed to the recent reviews of Chaudhuri (1976), Shehadeh (1972), and Yamazaki (1976).

Drawbacks to the technique of hypophysation may be grouped as problems of dosage and problems of supply. The problem of dosage is largely an outcome of the crudity of the technique: the activity of an extract depends upon the age, sex, and state of maturity of the donor, as well as the method of collection and the technique used to preserve the pituitary (Jalabert et al. 1977). Furthermore, numerous pituitary hormones unconnected with reproduction are present in the extract, making estimation of its specific activity a matter of guesswork. The phylogenetic distance between donor and recipient is another factor that renders the calculation of dosage largely empirical. The usual practice is to increase the dosage for heteroplastic pituitaries; pituitaries from immature marine catfish have, for example, been administered at five times the homoplastic dosage to induce ovulation in Indian carp (Varghese et al. 1975). Although the dosage administered was high, the cost of collecting and processing the catfish pituitaries was only one-fifth that for the homoplastic donor; because the catfish are split and sun-dried before being marketed, removal of the pituitaries — a procedure that mutilates the head — does not affect marketability.

This observation serves to introduce the problem of pituitary supply. Removal of the gland involves the sacrifice of a potential breeder (though it may also be carried out with spawned fish), and the collection of pituitaries from fish going to market may, in many parts of the world, seriously devalue the product. Though a suitable substitute may be found, the demand for pituitaries generally exceeds the supply, and collection is time consuming. It is fortunate, therefore, that pituitaries may be dehydrated, ampouled, and stored almost indefinitely, and recent years have seen the establishment of pituitary banks, notably by the Indian Central

Inland Fisheries Research Institute and by the FAO/UNDP Aquaculture Development and Coordination Program in Rome (Anonymous 1977d).

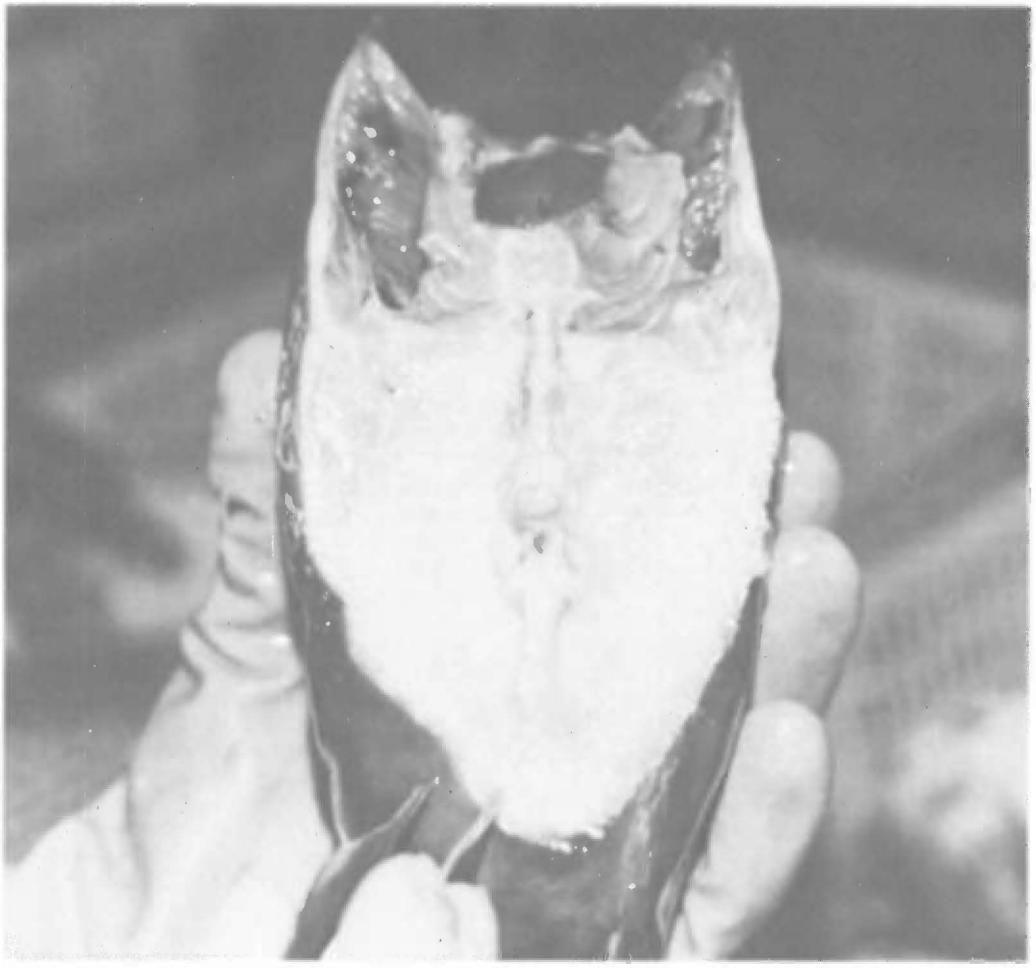
Standardization of dosage cannot, however, be achieved without a knowledge of the gonadotropic activity of the extracts, and the development of a suitable assay therefore assumes considerable importance. Teleost gonadotropins are inactive in most nonfish bioassays (Burzawa-Gerard and Fontaine 1972); attempts to arrive at a suitable procedure have been reviewed by Burlakova and Belyanov (1972) and Shehadeh (1972). An assay based on the uptake of radioactive phosphate by testicular tissue in the day-old chick has been used for several years by Donaldson and coworkers and has proved sensitive and precise (Donaldson 1973).

Effort continues to be directed toward finding a purified gonadotropin whose activity may be standardized and whose supply may be ensured as a replacement for crude pituitary extracts. Partially purified gonadotropin from the chinook salmon *Oncorhynchus tshawytscha* (Donaldson et al. 1972) has been used to induce ovulation in several cultured species, among them catfish (Sundararaj et al. 1972). and mullet (Kuo and Nash 1975). The average cost ($120 Canadian per injection) is, however, very high. In Malaysia, attempts to induce ovulation in bighead carp *Aristichthys nobilis* with partially purified salmon gonadotropin (SG-G100) produced only occasional success and only at dosages (14 mg/kg) comparable with the effective dose of acetone-dried common carp pituitaries; the combined effects of species specificity and cost were found to be prohibitive (Tajuddin 1978 unpublished).

Cost is less of a factor with several gonadotropins of mammalian origin. These hormones are produced by the placenta, and two are available in purified form: human chorionic gonadotropin (HCG) extracted from the urine of pregnant women and PMS from pregnant mare serum. The former has assumed by far the greater importance in fish culture. Its physiologic action resembles that of mammalian luteinizing hormone; however, the two hormones are not structurally identical, and there exists considerable species variability in response to the placental factor. The effect of these mammalian hormones is in general more uncertain than that of piscine gonadotropins (Fontaine 1976), and the uncertainty probably reflects the considerable phylogenetic distance between the two groups. The fact that effective doses may be cheaper than those of fish pituitary material makes their use attractive (Carreon et al. 1976). Human chorionic gonadotropin (HCG) has been widely employed in spawning cultured fish (Pickford and Atz 1967; Yamazaki 1965), and its successful use has generally been attributed to its LH-like activity (Chaudhuri 1976). Either alone or in combination with mammalian pituitary extract (a commonly used preparation is Synahorin), HCG has proved effective and economical in inducing spawning in the major Indian carps (Chaudhuri 1976; Bhowmick 1979), the grey mullet *Mugil cephalus* (Liao 1974), and the Chinese carps *Aristichthys nobilis* and *Hypophthalmichthys molitrix* (Tajuddin 1978 unpublished), when administered along with homo- or heteroplastic pituitary extract. There are few instances of its successful use alone, although Chen et al. (1969) and Carreon (1977) report ovulation under experimental conditions in the major Chinese carps and the catfish *Clarias macrocephalus*, respectively. Kuo et al. (1973) report natural spawning in mullet in response to a priming dose of 20 IU/g, followed 24 hours later by an injection of 40 IU/g.

Little research effort has been directed toward assessing the influence of temperature on the effectiveness of administered gonadotropin. It is, however, well known to fish culturists that an increase in water temperature decreases the delay between injection and ovulation (chapter 4). Stacey and coworkers (1979, in press) have shown that, in goldfish induced to ovulate through injection of HCG or salmon gonadotropin (SG-G100), the latency between injection and ovulation is highly temperature-dependent, with each 4–5 °C increase significantly reducing the delay to egg release.

The technique of hypophysation, despite its apparent sophistication, remains an art. It is unlikely to be displaced as the method of choice for the rural fish farmer, and indeed it may be carried out in the absence of such amenities as refrigeration, centrifuge, and electrical balance (Bhowmick and Kowtal 1973). Until a substance fulfilling the requirements for low cost, known activity, and ease of storage becomes generally available, the culturist's success will be augmented more by the development of a reliable method of ascertaining the state of gonadal development than by ad hoc chemical manipulation. There are, however, encouraging signs that an appropriate agent may soon be available. Acetone-dried salmon pituitary powder is now being commercially produced by Syndel Laboratories Ltd, Vancouver, BC; this preparation has the advantages of low cost and known activity and may assume considerable importance in future breeding programs throughout Southeast Asia.



Section of a chum salmon head showing the location of the pituitary with the mid-brain folded back to expose it for extraction.

GONADOTROPIN–RELEASING HORMONES (STAGE 3, FIG. 3)

The introduction of the synthetic decapeptide LH-RH (luteinizing hormone releasing hormone) has made possible intervention in the endocrinological chain at a point one step removed from the application of gonadotropin itself: namely, at the hypothalamic–pituitary interface. Work in this area is comparatively recent, and results obtained are largely experimental. The field is, however, a promising one, and future developments will almost certainly benefit the culturist.

The administration of synthetic LH-RH causes an elevated plasma level of gonadotropin hormone in the common carp *Cyprinus carpio* (Breton and Weill 1973) and in the male brown trout *Salmo trutta* (Crim and Cluett 1974); in the latter the effect has been noted in mature fish only. This action of LH-RH has also been demonstrated by intracerebral injection in the goldfish (Crim et al. 1976). Intracellular administration of the drug at a dosage of 1 μg/kg stimulated ovarian maturation in the common carp (Sokolowska et al. 1978), and indeed large doses of synthetic LH-RH have induced ovulation in goldfish (Lam et al. 1975; 1976) and in the ayu *Plecoglossus altivelus* (Hirose and Ishida 1974). Lam and coworkers (1978 unpublished) have noted a seasonal change in the effectiveness of LH-RH in inducing ovula-

tion. The variation in mode of administration of the releaser — intraperitoneal, intracerebral, and intramuscular injection have all been tried — makes comparison of the results difficult, and a systematic study of this factor would be of value.

It has been suggested that the magnitude of the dose required to bring about ovulation in fish, relatively high by mammalian standards, reflects a phylogenetic specificity of the releasing hormone. Numerous synthetic structural analogues of LH-RH are, however, now available, and certain of them possess a longer half-life and are more potent in mammals than the decapeptide LH-RH (Arimura et al. 1974; Monahan et al. 1973). Recent reports of their use in fish strongly suggest an important future role in the control of fish reproduction. Lam and coworkers (1978 unpublished) have found the nonapeptide LH-RH analogue [D-Ala^6-des-Gly-NH$_2$^{10}]-LH-RH-ethylamide (Ayerst 25205) to be partially effective in inducing ovulation in the goldfish at a dosage (10 ng/g) ineffective for LH-RH, and Donaldson et al. (1978) found the same analogue to be more potent than LH-RH in inducing final maturation and ovulation in the coho salmon (*Oncorhynchus gorbuscha*).

Still greater potency has been demonstrated for the LH-RH analogue D-Ser-(But)^6 -des Gly^{10}-LH-RH ethylamide (Hoechst 766); doses as low as 0.011 mg/kg were effective in inducing ovulation in coho salmon, when preceded by a priming injection of salmon gonadotropin (Donaldson et al. 1979). Work on LH-RH analogues has been carried furthest in China, where the ability of Ayerst 25205 to induce spawning in the silver carp *Hypophthalmichthys molitrix*, the bighead carp *Aristichthys nobilis*, the grass carp *Ctenopharyngodon idellus*, and the black carp *Mylopharyngodon piceus* has been extensively investigated since 1974 (Anonymous 1977a, b). The compound (termed LRH-A) is administered either intramuscularly or intraperitoneally, with the minimum effective dosage found to be 1 μg/kg and 0.002 μg/kg respectively. In grass carp, which earlier experience had shown to be refractory to HCG alone, a single injection of 5–10 μg/kg LRH-A produced an ovulation rate of 86%, comparing with results obtained using carp pituitary. For silver and bighead carp the minimum dosages were 3 μg/kg and 1.4 μg/kg, delivered in two injections; for black carp a dosage of 5–200 μg/kg, followed by 0.5–2 mg pituitary extract, produced a spawning rate of 74.6%. It is not clear whether natural spawning occurred or whether the fish were stripped. LRH-A was also effective in inducing spermiation in ripe males. An unexpected benefit was the effect of LRH-A on overall mortality in breeders: in 1974, using HCG and/or carp pituitary extract, the postspawning mortality was 57%; in 1975 and 1976, when LRH-A was used, mortality dropped to 11.8% and 3.5% respectively. Further experimentation is necessary before this decrease may be wholly attributed to the change in hormone.

ADMINISTRATION OF SEX STEROIDS (STAGE 4, FIG. 3)

Gonadotropin, whether endogenous or exogenous, is believed to stimulate the biosynthesis of the steroid 17α-20β Pg in the follicular envelope; this steroid then induces final maturation of the ova. Estrogens, though playing a role in the earlier stages of oocyte development, particularly vitellogenesis, do not appear to be involved in the later stages of maturation — the particular concern of the culturist. Chemical intervention at the level of the oocyte itself has some intuitive appeal; it appears to represent a simpler solution in which such hormonal intermediaries as gonadotropin and releasing hormone are eliminated. There exist, however, very few reports of the successful induction of ovulation through the use of sex steroids.

Induction of ovulation in goldfish following administration of progesterone has been claimed by Khoo (1974); estrogens, androgens, and pregnenolone were ineffective. More success has been had with 17α-20β Pg, demonstrated by Jalabert (1976) to be a potent inducer of in vitro oocyte maturation in goldfish, rainbow trout, and northern pike. Although Lam and coworkers (1978) failed to induce ovulation in the goldfish with this substance, Jalabert et al. (1977) found it effective in *Cyprinus carpio* following priming with a low dose of pituitary extract. It appears that injection of 17α-20β Pg is effective only during the late stages of oocyte maturation. This imposes a requirement for strict timing that appears to limit its potential practical application, particularly in the absence of a reliable method for assessing the state of female gonadal maturation (ovarian biopsy).

A second, less direct avenue is through the feedback control of gonadotropin secretion. It is believed that the release of gonadotropin is adjusted through a system of negative feedback, in which centres in the pituitary and hypothalamus are responsive to the level of circulating gonadal steroids (Peter and Crim 1979). A rise in the level of sex steroids, for example, brings about a decrease in gonadotropin secretion, with the result that steroid release again falls to the appropriate level; a drop in the steroid level has the opposite effect (Fig. 5A). Evidence for this

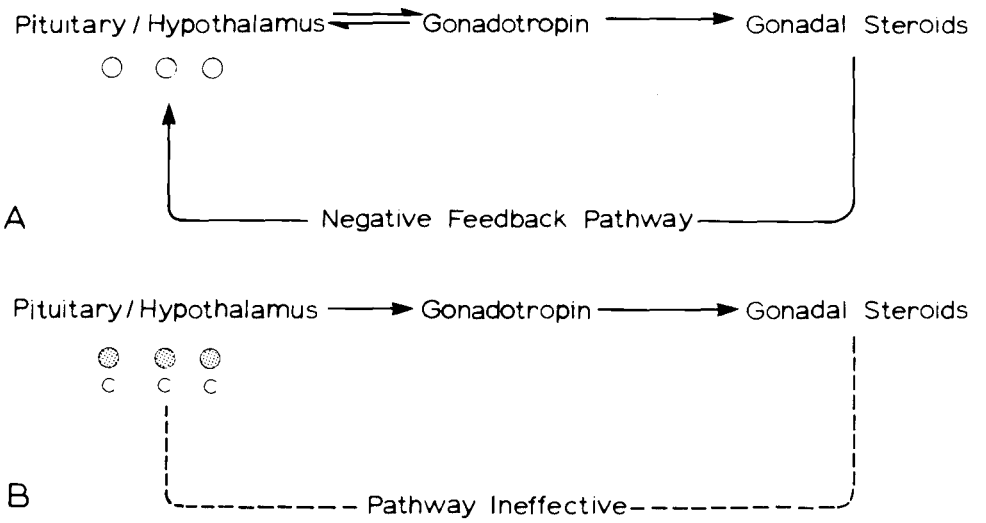


Fig. 5. *The control of gonadal steroid level: (A) the system operates normally, and the output of gonadotropin is adjusted as a result of the interaction of sex steroids with binding sites in the pituitary and hypothalamus (open circles). (B) the binding sites are occupied by clomiphene molecules, with the result that gonadotropin release is uncontrolled, and the level of gonadal steroids continues to rise.*

kind of control system is provided by Billard et al. (1977) who report increased plasma gonadotropin levels after castration in rainbow trout, and the responsiveness of the pituitary and hypothalamus to gonadal steroids has been shown by their uptake in these areas in the platyfish *Xiphophurus maculatus* and in the goldfish (Kim et al. 1978). These centres may be thought of as steroid-binding sites, and effort has recently been directed toward taking advantage of their sensitivity to gonadal steroids as a means of artificially stimulating gonadotropin release.

What is required is a compound that competes with endogenous gonadal steroids for binding sites in the pituitary and hypothalamus so that gonadotropin is released regardless of the level of the hormones. A negative feedback system becomes, then, a positive one, and results in an artificially elevated level of gonadal steroids (Fig. 5B). A chemical believed to possess this property is the antiestrogen clomiphene citrate (Pandey and Stacey 1975). Increased plasma gonadotropin levels have been produced by the implantation of clomiphene in the pituitary of goldfish (Billard and Peter 1977), and this gonadotropin surge may be responsible for the induction of ovulation in goldfish known to be caused by the drug (Pandey and Hoar 1972).

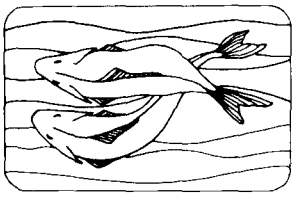
The choice of the dosage of clomiphene sufficient to induce ovulation remains a problem — Breton et al. (1975a) note that, in the common carp, a dose in the range of 1 mg/kg provokes a release of gonadotropin, whereas dosages much greater than 10 mg/kg have the opposite effect. Response to the drug is, in addition, dependent upon the state of gonadal maturation. It is for reasons such as these that practical use of clomiphene and other antiestrogens such as tamoxifen (Donaldson et al. 1978) awaits future research developments. Specific applications have, however, been suggested: Abraham (1975) notes that gonadotropin-secreting cells in the mullet *Mugil cephalus* reared in freshwater exhibit decreased synthetic activity in comparison with those from fish reared in seawater and suggests that the accompanying arrested ovarian development could be overcome by the administration of clomiphene. In one of the few field trials reported to date, attempts to induce ovulation in the Indian carp *Labeo rohita* with the drug were unsuccessful (Bhowmick et al. 1979).

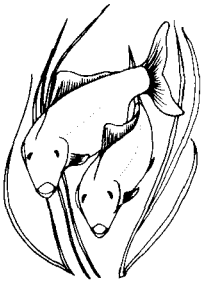
NEW AVENUES

The complexity of endocrinological interrelationships makes it difficult to state unequivocally whether the observed effect of an administered hormone is direct, or whether, as is the case with gonadotropin, it is mediated by secondary hormonal links. The situation becomes even more complicated when the administered substance is

itself a mixture of hormones, and this is the case with the crude pituitary extract used for hypophysation. The presence of thyrotropin, for instance, may contribute significantly to the effect on gonadal development generally (and loosely) ascribed to gonadotropin. Hurlburt (1977a, b) has shown that thyroid hormone may play an important role in ovarian maturation (chapter 2), and cycles in thyroid activity have been noted to coincide with gonadal maturation in some teleosts (Sage 1973). The role of the liver in vitellogenesis has already been noted, and thyroid hormone has been shown to have a regulatory effect on the liver in fish (Takashima et al. 1972). Further investigation of the functioning of the thyroid gland in teleosts may, therefore, indicate a role for thyroid hormone in the practical control of fish reproduction.

The possible importance of growth-promoting hormones should not be overlooked. The teleost ovary may increase from 1% of total body weight to 20% during maturation, and its energy requirements are large. Various growth-promoting factors may, therefore, play important roles in gonadal development, and the possible importance of these substances in fish culture should be reflected in future research programs. Both prolactin and growth hormone are produced by the teleost pituitary; the former has been implicated in reproduction and somatic growth in a variety of vertebrates (Hoar 1975). Effects of growth hormone administration in fish have recently been reviewed by Donaldson et al. (1978).





4. INDUCED BREEDING: PRACTICE

COMMON, CHINESE, AND INDIAN MAJOR CARPS

The literature concerning the artificial induction of ovulation in carp presents certain problems for the reviewer. Judged by international scientific criteria, it is, for the most part, of a low standard. It is common, for example, for species to be unidentified. A hypophysation procedure may be described for "Chinese carp" only — a description of little use to the reader who is attempting to spawn grass carp and who already knows that this species is generally more difficult to spawn than silver or bighead carp. The origin of injected pituitary extracts is often not clear, and a report may not state whether the glands are from male or female fish, mature or immature. Rarely is "wet weight" or "dry weight" specified when the calculation of dosage is described; yet this information is indispensable to the culturist attempting to adopt a new procedure. Reports of "successful" spawning induction are seldom the result of controlled experiments, and the criteria used to describe the degree of success achieved are often unsuitable. The term "positive response" is frequently encountered; yet it is not clear whether this refers to ovulation, spawning, fertilization, or hatching. An important variable that makes the comparison of results difficult is the method employed for egg incubation; this is a major determinant of fry survival, yet specifics are in many cases not given.

This criticism is not intended to be pejorative for there are underlying causes that make the literature difficult to interpret. To begin with, much of the research is carried out by practical culturists who have not received formal scientific training. The facilities available for experimentation are usually those of a fish culture station, often in a "developing" region of the world in which scientific equipment is difficult to obtain. A third, less tangible reason is that the practice of hypophysation may have assumed some of the characteristics of an art, with the result that most published reports have a distinctly regional flavour; it is as though each technique represents an addition to the local repertoire, with little attention paid to the underlying biologic reasons for success or failure.

Basic biologic laws are, however, inescapable, and the problem of inducing ovulation remains a scientific one. As such it is best tackled through properly designed experiments performed under controlled conditions. There is no reason that research of this kind cannot coexist with less rigorous reports from field stations; the important thing is that it be initiated. That this sort of program can yield important results in a comparatively short time is borne out by the research on induction of ovulation in mullet and milkfish. Work on these species, though recent, has been largely localized in well-equipped installations in Hawaii, Taiwan, and the Philippines, and the experimental design made possible in these laboratories has produced results of a higher calibre.

The same species of fish may be cultivated in different parts of the world, and its rate of growth and reproductive development may vary with the climatic zone. Chinese carp, for example, are an "exotic" species when cultured in India and may reach sexual maturity in half the time required in the native region. For this and other reasons, it is difficult to present a "standard method" for hormonal induction of ovulation in any of the Chinese or Indian major carps. The approach adopted here has, therefore, been to assemble a comparative table of published methods that have demonstrated some degree of success (Table 1). The list is selective, not, it is hoped, arbitrary. The data come in most cases from the published results of breeding experiments, although the procedures recommended in various manuals of fish breeding are included where appropriate. Common, Chinese, and Indian major carps are grouped by species, and the country in which breeding took place is included for purposes of comparison. Given the present state of experimentation in this field, the reader may regard the table as a source of ideas rather than a list of definitive methods.

TIME OF YEAR; AGE OF BREEDERS

It has long been known that temperate species grow faster and achieve sexual maturity sooner when cultivated in a tropical climate. Chinese

Table 1. Induced breeding techniques — common, Chinese, and Indian major carps.

Species (country)	Time of year	Age of breeder (y)	Number of females	Water temp (°C)	Assessment of gonadal state	Injection			
						Substance (route)	Solvent	First dose	Second dose
Cyprinus carpio (Nepal)	–	–	–	25–28	F: swollen, soft belly; cloaca swollen and reddish M: running milt on gentle pressure	Acetone-dried *Cyprinus carpio* pituitaries (IM)	0.6% NaCl: glycerine, 7:3	F: 1 pituitary/kg weight M: 1 pituitary total	–
Cyprinus carpio (Singapore)	–	–	13	24–27	F: belly swollen; pinkish cloaca M: running milt on gentle pressure	*Puntius gonionotus* pituitaries (IM)	0.6% NaCl or distilled water	F: 6 mg/kg wet weight	F: 6 mg/kg M: 12 mg/kg
All Indian major carps (India)	May–July (monsoon)	2–4	–	24–31	F: soft, bulging abdomen; swollen, pink vent M: running milt on gentle pressure	Homoplastic pituitaries (IM)	0.3% NaCl	F:2–3 mg/kg	F: 5–8 mg/kg M: 2–3 mg/kg
Labeo rohita (India)	July–Aug	–	32	–	–	Homoplastic pituitaries from donor of comparable size (IM)	Distilled water	F: 0.5 gland in 0.5 ml water	F: 1 gland in 0.75 ml water M: 0.25 gland in 0.5 ml water
Labeo rohita, Cirrhinus mrigala (India)	July	2–4	11	25–27	–	*Tachysurus* sp. pituitaries — marine catfish (IM)	–	F: total of 30 mg/kg M: total of 20 mg/kg	–
Hypophthalmichthys molitrix (Israel)	–	2–4	60	–	F: enlarged, soft belly M: milt on gentle pressure	*Cyprinus carpio* pituitaries (IM)	0.75% NaCl	–	–
Hypophthalmichthys molitrix (Malaysia)	–	2	12	27–28	F: full, soft belly; swollen, pink cloaca M: milt on pressure	M or F *Cyprinus carpio* or *Puntius gonionotus* pituitaries (IM or IP)	0.6% NaCl	F:5–6 mg/kg wet weight	F: 5–6 mg/kg M: 5 mg/kg
Ctenopharyngodon idellus (Malaysia)	–	2	10	27–28	–	M or F *Cyprinus carpio* or *Puntius gonionotus* pituitaries (IM or IP)	0.6% NaCl	F: 5–6 mg/kg wet weight	F: 5–6 mg/kg M: 5 mg/kg
Ctenopharyngodon idellus (China)	–	–	25	20–28	–	LRH – A[b]	Water base	F: 5–10 μg/kg M: 2.5–5 μg/kg	–
Ctenopharyngodon idellus (India)	July–Aug	–	11	28–31	–	Homoplastic and heteroplastic pituitaries (IM)	–	F: 3–5 mg/kg	F: 7–10 mg/kg M: 2–3 mg/kg
Ctenopharyngodon idellus (Thailand)	July–Oct	1–2	8	27–33	–	*Cyprinus carpio* pituitaries plus Synahorin (IM)	0.6% NaCl or distilled water	F and M: 0.23–1 pituitary	F: 1–3.4 pituitaries plus 20 rabbit units Synahorin

(Table 1 con't.)

ΔT^a (h)	Delay to ovulation	Fertilization method	Incubation method	Results	Notes	Reference
–	8–10	Stripped; dry	Cloth-walled hatching funnel or *hapa*; 5–10 l water/min	Instruction manual; no results given	Delay to ovulation increases with decreasing water temperature. Contains information on hypophysation of Chinese carps, but injection details are unclear; considerable information on fertilization and incubation techniques	Woynarovich (1975)
5	6–7	Natural spawning	–	All spawned; 65 000 fry obtained	Exact quantity of pituitary extract required has not yet been determined	Tay (1973)
6	3–6	Natural spawning in breeding *hapa*	Stagnant double *hapa*; eggs removed from breeding *hapa* 6–8 h after fertilization	Instruction manual; quotes 60–100% success	This "standard method" for Indian carps has undergone little modification since 1963. Best results are obtained on cool, windy, rainy days	Chaudhuri (1972)
6	–	Natural fertilization	–	72% positive results; 1 650 000 eggs produced	A simplified version of the preceding method, intended for use by the rural fish farmer. Extract is prepared just before use; it is not centrifuged	Bhowmick and Kowtal (1973)
–	–	–	–	Positive response in 7 of 11 females	Dosages of 20 mg/kg (female) and 15 mg/kg (male) ineffective. High dosages used in view of phylogenetic distance between donor and recipient. Cost is 1/3 that of homoplastic pituitaries	Varghese et al. (1975)
–	–	Stripped; dry	Incubators with flowing water	3 million larvae hatched; 50% survival to 0.25 g stocking size	Injection carried out while fish in water to eliminate trauma	Pruginin and Cirlin (1976)
5	5–6	Stripped; dry	In hatchery: trays with running water; in ponds: stagnant *hapas*	Hatchlings in excellent condition for 30% of females injected	Addition of Synahorin to extract has little effect	Chen et al. (1969)
5	5–6	Stripped; dry	In hatchery: trays with running water; in ponds: stagnant *hapas*	Hatchlings in excellent condition for 40% of females injected	State of maturity of female cannot be assessed by gut shape due to presence of mesenteric fat	Chen et al. (1969)
–	15–20	–	–	88% spawning	Total 139 fish treated with LRH-A under various injection regimens, with a resulting overall spawning rate of 86.3%	Anonymous (1977 a, b)
–	5–6	Stripped; dry	Stagnant *hapas*	15–80% fertilization; 550 000 fry produced	Combining Synahorin (25 IU) with pituitary extract proved ineffective	Singh et al. (1970)
6–8	4–6	Stripped; dry	*Hapas* in running water	47% ovulation	–	Boonbrahm et al. (1968)

(con't.)

(Table 1 con't.)

Species (country)	Time of year	Age of breeder (y)	Number of females	Water temp (°C)	Assessment of gonadal state	Injection			
						Substance (route)	Solvent	First dose	Second dose
Ctenopharyngodon idellus (USA, Arkansas)	May–June	–	9	23–25	F: abdomen distended M: milt on pressure	Homoplastic acetone-dried pituitaries (IP) plus HCG (IM)	–	F: HCG 45 IU/kg	F: 383 IU HCG/kg, followed by 0.45 mg/kg pituitary in 24 h (3 injections)
Ctenopharyngodon idellus (Nepal)	May–June	4	12	20–28	F: pinkish vent M: milt on pressure	Homoplastic pituitaries	–	F: 0.5–3 mg/kg dry weight	F: 2.5–3.5 mg/kg
Hypophthalmichthys molitrix (Malaysia)	–	–	–	25–27	–	HCG plus *Cyprinus carpio* pituitaries	–	F: 200 IU HCG/kg	F: 4 mg/kg *Cyprinus carpio* pituitary
Hypophthalmichthys molitrix (China)	–	–	198	20–28	–	LRH–A[b]	Water base	F: 10 μg/kg M: 5 μg/kg	–
Hypophthalmichthys molitrix (India)	July–Aug	–	40	28–34	–	Homoplastic and heteroplastic pituitaries (IM)	–	F: 4 mg/kg	F: 8–10 mg/kg M: 2–3 mg/kg
Hypophthalmichthys molitrix (Thailand)	May–Aug	1–2	10	27–33	–	*Cyprinus carpio* pituitaries plus Synahorin (IM)	0.6% NaCl or distilled H_2O	F and M: 0.23–1 pituitary	F: 1–3.4 pituitaries plus 20 rabbit units Synahorin
Aristichthys nobilis (Malaysia)	–	2	18	27–28	F: full, soft belly; swollen, pink cloaca M: milt on pressure	M or F *Cyprinus carpio* or *Puntius gonionotus* pituitaries (IM or IP)	0.6% NaCl	F: 5–6 mg/kg wet weight	F: 5–6 mg/kg M: 5 mg/kg
Aristichthys nobilis (Malaysia)	–	–	–	25–27	–	HCG plus *Cyprinus carpio* pituitaries	–	F: 200 IU HCG/kg	F: 4 mg/kg *Cyprinus carpio* pituitary
Aristichthys nobilis (China)	–	–	108	20–28	–	LRH–A[b]	Water base	F: 1–2 μg/kg M: 0.5–1 μg/kg	F: 8–9 μg/kg M: 4–4.5 μg/kg
Aristichthys nobilis (Thailand)	Aug–Sept	1–2	5	27–33	–	*Cyprinus carpio* pituitaries plus Synahorin	0.6% NaCl or distilled H_2O	F and M: 0.23–1 pituitary	F: 1–3.4 pituitaries plus 20 rabbit units Synahorin

[a] T = time between first and second injections

[b] LRH–A = [D-Ala[6]–Des–Gly–NH_2[10]]–LH–RH-ethylamide

carps are a notable example, and they have been successfully reared in areas as widely separated as Russia and Malaysia. In Moscow, for example, the Chinese carps reach sexual maturity at age 10, whereas they do so in only 2 years in Malaysia. Elevated water temperature and increased photoperiod may be considered to be the primary factors responsible for accelerated maturation in warmer climates, although the influence of diet should not be discounted; Bienarz and coworkers (1977) demonstrated accelerated ovarian maturation in specimens of *Cyprinus carpio* fed a protein-rich diet, and supplementary feeding has been shown to produce the same effect in the Indian carp *Labeo rohita* (Bhowmick et al. 1977).

After sexual maturity has been reached, the ovaries of the major Chinese and Indian carps, at any given time, contain oocytes in several stages of development. For this reason it is possible that, under the appropriate conditions of temperature and diet, individuals may produce ripe ovulated eggs more than once a year, especially in tropical countries in which unchanging high water temper-

(Table 1 concluded)

ΔT^a (h)	Delay to ovulation	Fertilization method	Incubation method	Results	Notes	Reference
24	10–16	Stripped; dry	In hatchery: jars with running water	66% ovulation; 20–98% hatching	Fish anesthetized in Quinaldine 7–10 ppm for injection and stripping	Bailey and Boyd (1970)
6	12–14	Stripped; dry	Hatching funnels (20–30 l capacity) in running water (0.5–0.8 l/min)	11 of 12 females ovulated; 400 000 fry produced	No males injected since milt oozing freely	Shrestha (1973)
6	6–7	–	–	80% successful ovulation	Preliminary progress report, IDRC	Tajuddin (1978 unpublished)
–	22–24	–	–	84% induced ovulation	–	Anonymous (1977 a, b)
–	4–5	Stripped; dry	Stagnant *hapa*	5–80% fertilization; 907 000 fry obtained	Addition of 25–50 IU Synahorin to both female injections also effective. Maximum fry yields associated with rain and cool weather. Best water temperature 27–30 °C	Singh et al. (1970)
6–8	4–6	Stripped; dry	*Hapas* in running water	67.7% ovulation	–	Boonbrahm et al. (1968)
5	5–6	Stripped; dry	In hatchery: trays with running water; in ponds: stagnant *hapas*	Hatchlings in good condition for 66% of females injected	Addition of Synahorin to pituitary extract has little effect	Chen et al. (1969)
6	6–7	–	–	80% successful ovulation	Preliminary progress report, IDRC	Tajuddin (1978 unpublished)
12	–	–	–	82% induced ovulation	–	Anonymous (1977 a, b)
6–8	4–6	Stripped; dry	*Hapas* in running water	91% ovulation	–	Boonbrahm et al. (1968)

atures provide excellent conditions for fish growth. Chen et al. (1969) report that two or three spawning cycles may be encountered in a single year in Chinese carps reared in Malaysia and suggest that, by manipulation of diet, it should be possible to stagger and control the cycles. Recent results from Malacca indicate that this is feasible; Tajuddin (1978 unpublished) reports that it is now possible to spawn bighead and silver carp virtually every month of the year.

In countries such as India, which experience definite dry and wet (monsoon) seasons, the number of spawnings attainable in a single year is not so high as in the tropics, although Bhowmick et al. (1977) have recently succeeded in breeding the same specimens of the Indian major carps *Labeo rohita* and *Catla catla* twice in the same season. The Indian carps, in their natural habitat, spawn once a year. This takes place during the monsoon season, and the increased rainfall and lowered water temperature are believed to be the factors responsible for bringing about final gonadal maturation. As may be seen from Table 1, induced breeding is also carried out during these conditions.

ASSESSMENT OF GONADAL STATE

Success in hypophysation is dependent upon accurate assessment of the state of gonadal maturation in the female breeder. Ripe males are generally easily recognized by the production of milt upon slight pressure to the abdomen, and, as has already been noted, hypophysation of males is often unnecessary. Published studies of carp breeding have relied on external characteristics such as "swollen belly" and "pinkish vent" for the determination of the state of gonadal maturation in females; yet this method relies upon the subjective interpretation of colours and shapes and is often unreliable. A full belly may, for example, reflect a distended gut, and a swollen and pinkish genital pore is commonly found in fishes whose ovaries have already begun to regress (Chen et al. 1969). The method is particularly difficult to apply to grass carp, and it is probably for this reason that, among the Chinese carps, this species is the most difficult to spawn by hypophysation. Success in the induced breeding of all cultured fishes will improve dramatically upon the development of safe and reliable methods of ovarian biopsy, and there is an urgent need for research in this area. A fuller treatment of this subject may be found in chapter 5, Methods of Ovarian Biopsy, where existing techniques are reviewed for carp, mullet, milkfish, and catfish.

INJECTION

Substance: Most of the methods summarized in Table 1 involve the injection of crude fish pituitary extract. In cases where heteroplastic pituitaries are used, the phylogenetic distance between donor and recipient is not great; the common carp, for example, is often employed as donor for both Chinese and Indian carps. The use of immature marine catfish pituitaries to spawn Indian carps should, however, be noted, particularly as this allows a considerable financial saving (Varghese et al. 1975). The use of the synthetic hormone LRH-A for spawning Chinese carps has already been discussed; further research may indicate an important role for this compound in fish culture operations throughout the world.

Crude fish pituitary extracts may originate from freshly dissected glands or from glands preserved in acetone or absolute alcohol. The available reports do not always identify the source of the gland, nor the "dry weight" or "wet weight" of the pituitary specified. Repetition of the reported experiments is therefore difficult. The solvent used is often similarly unspecified, although 0.6% NaCl appears to give consistent results. No more than 0.5 ml should be injected, and any insoluble particles remaining after maceration of the gland should be removed through either centrifugation or settling.

Determination of dosage: Ovulation is generally induced by the application of pituitary extract or gonadotropin in two doses. The first is termed "stimulatory" and the second "resolving"; males are usually injected at the time of the second injection to the female. The division of the total dosage appears to be somewhat arbitrary, and the time between injections is arrived at through trial and error. The method of dosage calculation reflects the equipment available to the culturist; the most consistent results are obtained when both the injected substance and the recipient breeder are accurately weighed and the dosage calculated on a weight/weight basis. Acceptable results may, however, be obtained in the absence of a balance; Bhowmick and Kowtal (1973) describe a simplified technique in which pituitary glands are taken from donors of a size comparable to the recipient. Weight of donor and recipient may be determined on the basis of a nomogram relating weight to measured length and girth (Makeyeva and Verigin 1970).

Route and method of injection: Intramuscular injection of hormones and extracts is by far the most commonly employed technique, although intraperitoneal administration is occasionally undertaken. The latter entails risk of damage to internal organs as well as possible loss of extract through inadvertent injection into the gut, and there appears to be little to recommend it. Intramuscular injection may be done between the base of the dorsal fin and the lateral line. The placement of the needle is not critical, but the culturist should lift scales carefully and massage the area gently following the withdrawal of the needle to aid distribution of the extract and to prevent backflow. Another means of preventing backflow is to increase the viscosity of the solution by adding glycerine to the extract in the ratio of 3:7 (Woynarovich 1975).

Handling of breeders: Removal of epidermal mucus in the course of handling a fish renders the skin susceptible to bacterial infection, as does more serious injury such as bruising, abrasion, and scale loss. Netting, injection, and stripping are all procedures involving considerable risk of injury, and care must be taken to ensure that the damage is minimal. A carelessly handled fish may not survive long enough to ovulate, whereas one that has been properly treated may be successfully bred for years.

The culturist should wrap the fish in a moist towel after netting and removal from the holding tank and the fish should never be thrown back into the water. An acriflavin bath (0.01%) has proved useful in the prevention of red scale injury commonly occurring when carp are spawned in captivity (Bhowmick et al. 1977) and should be applied following stripping. Anesthetics are employed only infrequently; yet their use could significantly increase survival. Both MS 222 (Woynarovich 1975) and Quinaldine at concentrations of 50–100 ppm (Chen et al. 1969) have been found effective for carps. MS 222 may be added to the water in doses of 50–100 mg/l, or a roll of cotton-filled cloth may be soaked in a 0.04M solution and inserted in the mouth of the fish. MS 222 (1.6 ppm) has successfully been used in the transport of grass and silver carp over long distances (Gupta and Sharma 1974). Information concerning general anesthetics commonly used for fish is provided by Bell (1967) and Anonymous (1978).

Injection may even be performed while the fish is in the water. This procedure eliminates the risk of internal injury that results when the internal organs are no longer supported by the surrounding water and may be carried out either on anesthetized fish or fish trapped in a net.

Delay to ovulation

Breeders are returned to their tanks following the final injection and, unless natural fertilization is desired, must be carefully watched for indications that the eggs and sperm are ready to be stripped. Readiness is most reliably forecast by courtship behaviour, although the female may be netted and gently massaged to ascertain whether her eggs have begun to flow freely. Loss of eggs may be prevented by suturing the genital opening at the time of injection; this must be done under mild anesthesia. There is little margin for error in the detection of a female that is ready for stripping. Eggs removed an hour too early may be infertile, and the Chinese carps in particular cannot be relied upon to spawn naturally, with the result that eggs that are not removed manually degenerate and become resorbed. This may occur as soon as 30 minutes after the eggs become fully ripe (Chen et al. 1969).

The delay between injection and ovulation varies with the number of injections and the water temperature, and an understanding of these influences enables culturists to arrange the schedule of injections so that stripping may be done at a convenient time of day. Delay following the last injection is markedly shortened when more than one injection is given and the time of ovulation is consequently more predictable. Considerable control may also be gained in areas where it is possible to alter the water temperature; in common carp, for example, ovulation occurs approximately 8 hours after injection at 28 °C, whereas three times that period is required at 15 °C (Woynarovich 1975).

Fertilization method

Fertilization may be accomplished either through manual stripping and mixing of eggs and sperm or through natural means in a spawning pond. Artificial fertilization is commonly done by the "dry" method: both breeders are dried, eggs are stripped into a dry container, milt is stripped over them, and the two are gently mixed with a feather or plastic spoon. The eggs are thoroughly washed with water after 1–2 minutes, then transferred to a larger volume of water (several litres) for swelling and water-hardening. Following this process, which is complete within approximately 1 hour, eggs may be transferred to an incubator or hatching device.

The eggs of common and Chinese carps are adhesive and may clump together and reduce the oxygen supply to the innermost eggs. This may be prevented by adding a solution of 0.4% NaCl and 0.3% urea to the eggs immediately following mixing of eggs and sperm (Woynarovich 1975; Kossmann 1976). The solution should first be added in about the same quantity as the volume of eggs and may be replenished, with stirring, as it is absorbed. Subsequent washing in a 0.15% tannin solution before removal to the incubator will remove any traces of adhesive material.

Natural spawning is commonly employed for the Indian carps and has the advantage of limiting the amount of handling the breeders receive. Disease and predation may, however, bring about an unacceptably low rate of fry survival if incubation of the eggs is in the spawning pond. For this reason fertilized eggs are generally collected after water-hardening and removed to special incubating devices or hatching enclosures. This must be done carefully and thoroughly if high survival rates are to be obtained.

Incubation method

Eggs that are fertilized through natural spawning and allowed to hatch in stagnant ponds are subject to predation and disease, and the percentage of hatching may be as low as 1–5. Some protection from predation is afforded by the traditional hatching *hapa*. This simple device (Fig. 6) consists

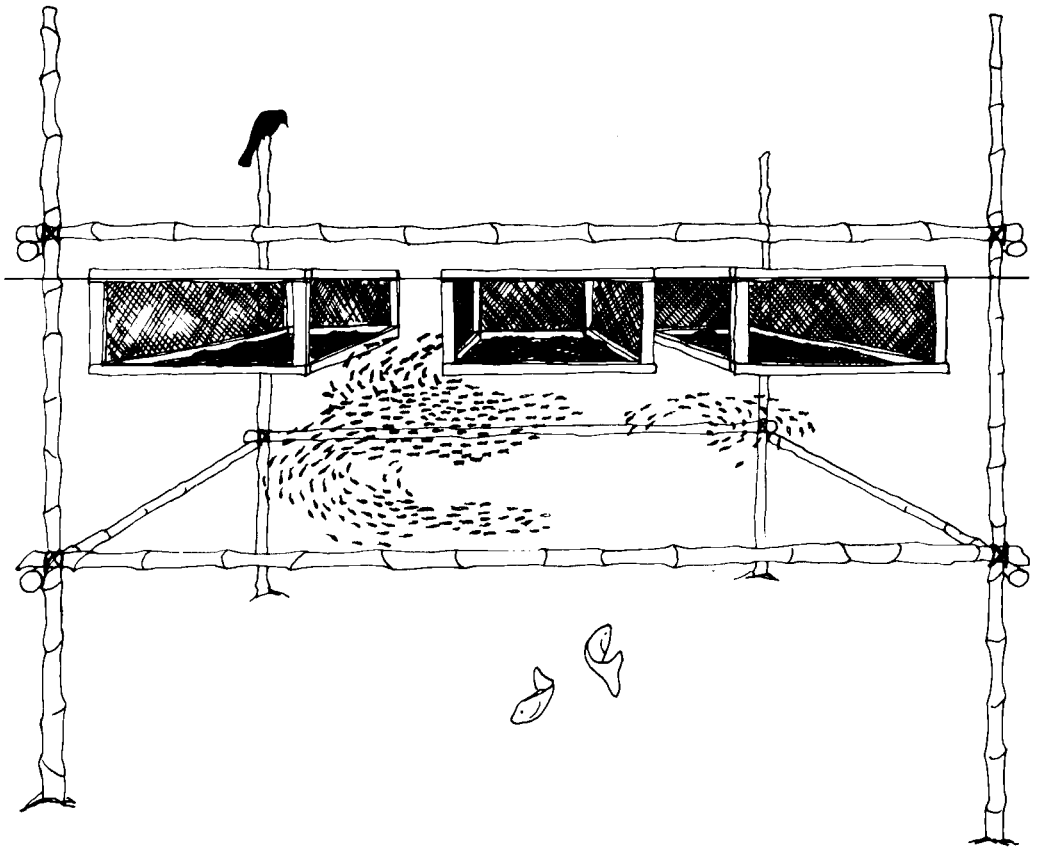


Fig. 6. *Diagram illustrating the principle of operation of a hatching* hapa. *The close-mesh netting surrounding the outer frame is omitted to show details of construction. Fry produced from eggs placed within the floating trays escape through the coarse mesh netting surrounding these containers but are unable to pass beyond the barrier of the outer* hapa.

of several shallow inner trays floated within a larger outer frame. The inner trays are covered with coarse netting such as mosquito netting, and the larvae produced from eggs placed within them will escape through the mesh, leaving dead eggs and eggshells behind. Larvae are retained within the outer *hapa*, which is covered with fine mesh netting. For best results, the hatching *hapa* should be set in running water, and predation from birds and frogs may be limited by providing a cover. Such a pond hatching device remains, however, subject to such uncontrollable hazards as temperature change and a drop in the water level, and for large-scale fry production an indoor hatching facility is required. A piped water supply is essential, for this enables the adjustment of flow and oxygenation as well as allowing the controlled application of agents to combat the spread of bacterial and fungal disease.

An indoor hatching apparatus may be of simple design; its main functions are to provide sufficient circulating water to maintain a slow rolling egg motion, to allow easy removal of such debris as dead eggs and egg shells, and to allow removal of the larvae for transfer to rearing ponds. A simple device suitable for incubating carp eggs is diagramed in Fig. 7. It has an 80-litre capacity and will hold up to 70 000 Chinese carp eggs. Water flow should be maintained at 1–2 litres/minute. The funnel may be constructed from galvanized iron or plastic; it may easily be fashioned from a plastic pail. In the simplest arrangement, the incubator is suspended from an overhead support, and water exits through the nylon mesh. Numerous refinements are possible: if the water flow is adjusted so that the slowly circulating eggs ascend only halfway up the funnel, the larvae may then escape through an outflow positioned over a

spillway. A shower or strainer device on the water inlet may provide better water circulation.

MULLET

The mullet, *Mugil* sp., is a particularly attractive species for aquaculture; it is widely distributed, temperature hardy, and omnivorous. It is, in addition, euryhaline and may therefore be stocked in fresh-, brackish, or saltwater. The collection of fingerlings from estuarine waters is, however, subject to unpredictable fluctuations in abundance, and recent years have seen considerable research directed toward assuring a supply of artificially reared stock.

ARTIFICIAL SPAWNING

Successful induction of ovulation in the mullet has been accomplished repeatedly within the last decade, although a method combining reliability with low cost has yet to appear. A variety of inducing substances has been employed, including carp pituitary homogenate (Yashouv 1969), mullet pituitary homogenate mixed with Synahorin (Liao 1975), partially purified salmon gonadotropin, SG-G100 (Shehadeh et al. 1973a), and human chorionic gonadotropin, HCG (Kuo et al. 1973).

Research on the spawning of the grey mullet *Mugil cephalus* has been carried out since 1963 in Taiwan by Liao and coworkers, and progress to 1973 has been summarized (Liao 1975). Two points concerning this work are especially noteworthy: the breeders were almost exclusively wild fish captured at the time of the annual spawning migration (December–February), and no objective attempt was made to assess the state of female gonadal development before injection. It has already been noted that the state of female readiness is critical to the success of any program of induced ovulation. The use of wild spawners also carries serious drawbacks; not only are they difficult to obtain from fishermen and women, but they are also commonly bruised and abraded during capture and have the added disadvantage of being unaccustomed to handling. Successful induction of spawning has, despite these difficulties, repeatedly been achieved using a combination of Synahorin and acetone-dried pituitaries from mature mullet. Pituitary gland or Synahorin alone were both found to be ineffective. The most successful total dosage was determined to be 2.5–6 pituitaries combined with 10–60 rabbit units of Synahorin, administered intramuscularly in two approximately equal injections 24 hours apart. The addition of vitamin E, 0–300 mg, is said to have increased the percentage of females responding: 71.4% of the females so treated ovulated successfully. Liao acknowledges the drawbacks of estimating dosage by numbers of whole pituitaries and suggests that improved results could be obtained by calculating dosage on a weight/weight basis. Attempts at natural fertilization were unsuccessful, and both male and female breeders were stripped. Dry and wet methods of fertilization were employed with equal success. It was found that hypophysation of males was unnecessary, and indeed this is a feature common to most of the published reports of mullet spawning in captivity.

A similar technique has been used with some success in India with *Mugil macrolepis* (Sebastian and Nair 1975). Again, wild spawners were used, and females were injected with a total of seven mature mullet pituitaries, delivered in two doses 6 hours apart. No ovarian biopsy was carried out, and natural fertilization was ineffective. Forty percent of the injected females are described as ovulating successfully. It is noteworthy that the dosages of pituitary material used in this study were very high; it is, however, significant that pituitaries were effective in the absence of such mammalian synergists as Synahorin and HCG.

The large-scale breeding of mullet is difficult to carry out when the supply of mature males and females is dependent upon natural sources. Studies carried out at the Oceanic Institute in Hawaii are,

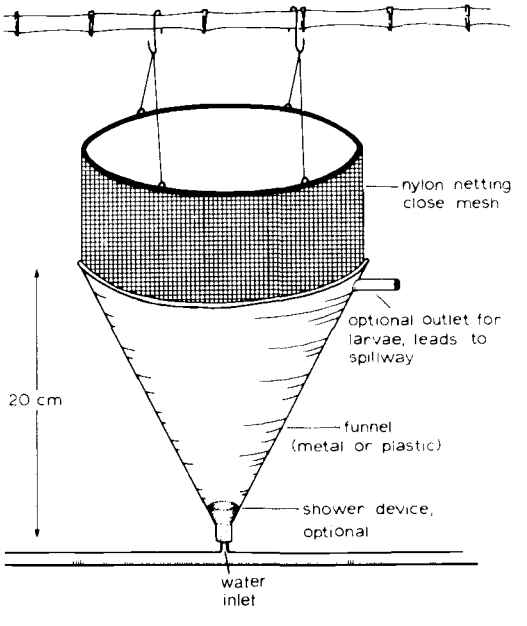


Fig. 7. *An incubator for carp eggs.*

therefore, significant in that breeders were obtained from pond-reared stock and spawned naturally in the breeding tanks. Consequently, there was no need for stripping (Kuo et al. 1974).

A second obstacle to success has been removed through the introduction of a safe and reliable method of ovarian biopsy (Kuo et al. 1974) (chapter 5). Ovulation was most reliably induced when mean oocyte diameter was greater than 0.6 mm; 0.65 mm was preferable. Best results have been obtained using partially purified salmon gonadotropin, SG-G100, administered intramuscularly in two injections: an initial dosage of one-third the total is followed in 48 hours by the remaining two-thirds. The relationship between the dosage of gonadotropin required to induce spawning and initial egg diameter of recipient is presented in Fig. 8, and the amount of hormone to be injected may be calculated directly from this graph. In instances where the average egg size is less than 0.6 mm, spawning may still be induced by daily injection of SG-G100 in doses increasing from 0.12–3.4 μg/g body weight. This procedure is carried out for 6–8 days, followed by hypophysation.

The high cost of SG-G100 makes this method inappropriate for many developing areas of the world. Human chorionic gonadotropin (HCG) is cheaper and more readily available; preliminary studies by Kuo and coworkers (1973) have shown that this hormone may be used alone to bring about ovulation in *Mugil cephalus*. The best procedure was found to be to inject a priming dose of approximately 20 IU HCG/g body weight when mean oocyte diameter had reached 0.6 mm, followed in 24 hours by a second dose of about 40 IU/g. Fertilization rates ranged between 45 and 98%.

Most research on the induction of ovulation in the mullet has been directed toward the initiation of spawning during the peak of the natural breeding season. In an attempt to prolong the breeding season of captive *M. cephalus*, Kuo and Nash (1975) have successfully induced ovarian maturation out of season through manipulation of photoperiod and temperature. It was found that the initiation of vitellogenesis, which normally follows a postspawning refractory period of 235 days, could be induced in as little as 56 days under a constant photoperiod of 6 hours light/18 dark, at a temperature of 21 °C. The "accelerated" fish could then be spawned by the injection of SG-G100. This suggests that grey mullet may, under controlled conditions, be spawned more than once in a single year and represents an important step in the use of environmental stimuli to induce gonadal maturation.

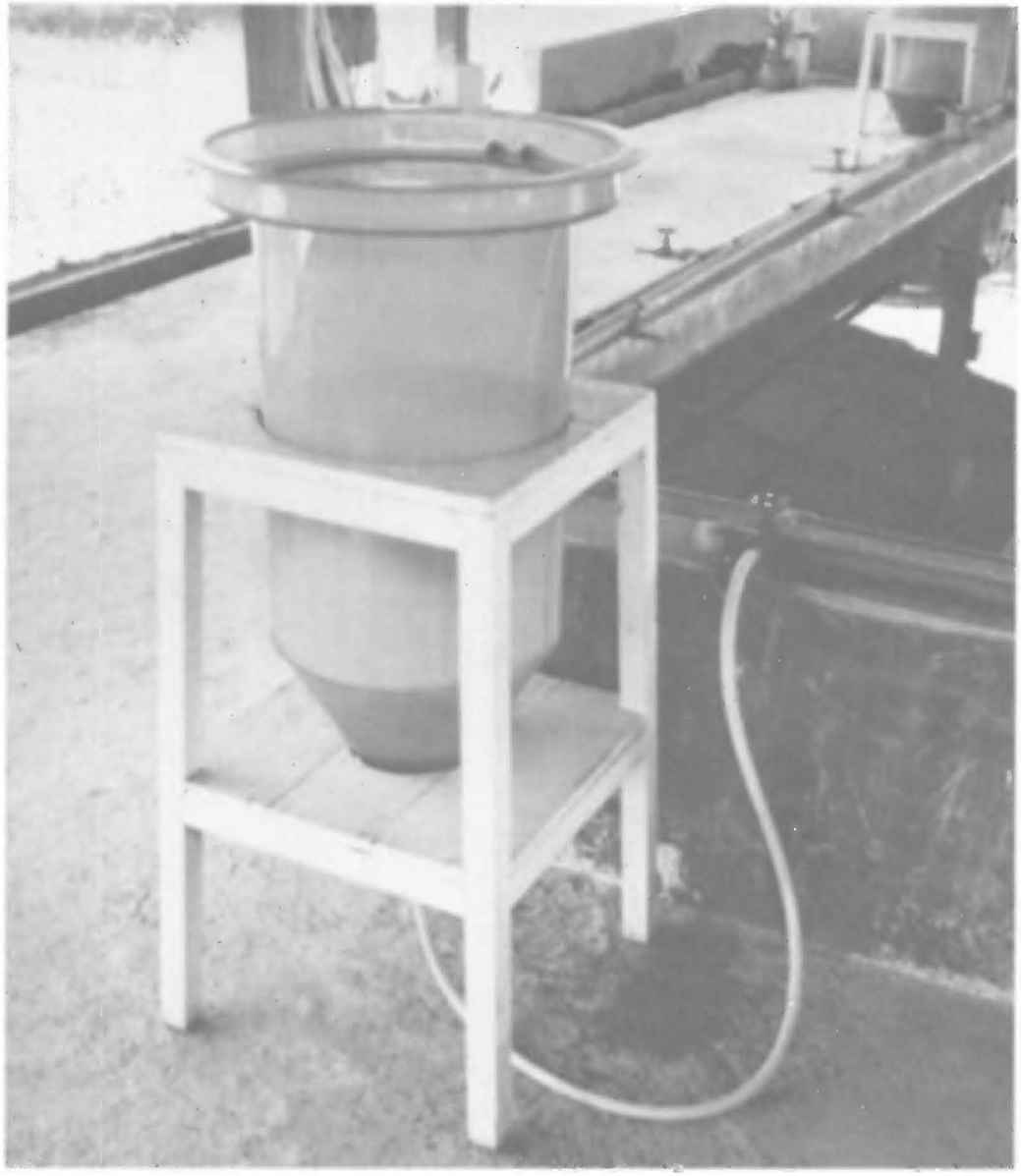
Larval Rearing

Although it is now possible to control the ovulation and spawning of several species of mullet, there remain several major stumbling blocks in the path toward the large-scale rearing of larvae. Whereas hatching rates may be high, survival through the first 42 days of development is, for most of the studies cited, less than 5%, with 20% the highest reported figure (Liao 1975). Mortality is generally associated with changes in the specific gravity of the larvae, which undergo two major descents in the water column: one at 2.5 days and the second at 8 days after hatching (Kuo et al. 1973). Mortality after the second descent is usually greater than that following the first and is often total. Nash and Kuo (1975) have reviewed the problems associated with the rearing of mullet and suggest that the freshwater content of larval food may contribute significantly to the phenomenon of sinking mortality, by influencing larval specific gravity. The importance of temperature, salinity, and diet is also stressed. Detailed procedures for the rearing of larvae of the grey mullet are provided by Nash et al. (1973); methods currently in use in the USSR (Anonymous 1976) and India (Sebastian and Nair 1975) are also of interest.

Milkfish

The milkfish *Chanos chanos* is a euryhaline species extensively cultivated in coastal brackish ponds and in enclosures in freshwater lakes in Indonesia, the Philippines, and Taiwan. It is abundant throughout the Indo–Pacific region, and efforts are being made to cultivate it for human consumption and for tuna bait in several other countries. Problems with induced breeding are in many ways similar to those encountered in breeding the mullet, and recent research on milkfish spawning has, in fact, been undertaken by groups previously concerned with mullet reproduction. Workers at marine research institutes in Taiwan and Hawaii, together with investigators at the Southeast Asian Fisheries Development Centre in the Philippines, have taken the first steps toward the production of fry under controlled conditions. It must be stressed that work in this field is just beginning and that reports are in general preliminary.

Breeding experiments with freshly captured milkfish present many of the same problems as do those with wild mullet. Handling stress is often poorly tolerated, although Vanstone et al. (1976a) report techniques for capture, transport, and domestication that have proved effective in reducing mor-



A simple, up-welling type incubator used for carp eggs in Thailand.

tality. Of greater significance is the fact that, of mature milkfish captured at sea, a large proportion are already spawned out and consequently useless for breeding experiments. Thus, it is essential that captured, spawned-out milkfish be maintained in captivity until they remature or that they be reared from young in captivity. The latter is not easy to accomplish. In Indonesia, for example, milkfish reared in large brackish water ponds had not attained sexual maturity at age 8 years (Alikunhi 1976). Indonesian and Philippine workers have begun programs of rearing 2-year-old fish in large tidal pools and floating enclosures using controlled diets in an attempt to overcome this refractoriness. Coastal impoundments stocked with yearling milkfish are also being considered as a source of spawners. Evidence that captive milkfish may mature sexually is, however, pro-

Table 2. Induced breeding — techniques for catfish.

Species (country)	Time of year	Age of breeder (y)	Number of females	Water temp (°C)	Assessment of gonadal state	Injection		
						Substance (route)	Solvent	Dosage
Pangasius sutchi (Thailand)	June–Sept	3	–	28–32	F: distended belly; pinkish vent; eggs loose, shiny yellow M: milt on pressure	Fresh or preserved catfish pituitaries (IM)	Distilled water	Not clearly stated: female second dose is 1.5–3 times the first; male given 1/4 the female dose at time of second female injection
Pangasius pangasius (Thailand)	Aug	5–6	2	27–31	–	*Clarias batrachus* pituitaries (IM)	0.8% NaCl	F: 3 injections given: 2, 6, and 12 pituitaries M: 2 pituitaries at time of first female injection
Clarias fuscus (Taiwan)	–	1.5–2	–	26–29	F: soft, distended belly; red genital pore	*Cyprinus carpio* pituitaries mixed with Synahorin	0.7% NaCl; 0.03% KCl; 0.026% $CaCl_2$; 0.003% $NaHCO_3$	F: 1 pituitary from donor 2–3 times the body weight of recipient, mixed with 20 rabbit units Synahorin; administered in 2 injections
Clarias macrocephalus (Philippines)	May–Sept	1	153	–	F: distended belly; red genital pore M: prominent, whitish genital papilla	HCG (IM)	–	F: single injection: 400–500 IU M: 150–250 IU
Clarias batrachus (India)	–	–	7	27–31	–	Indian carp pituitaries	–	80–90 mg/kg; no other information given

vided by Liao and Chang (1976), who raised naturally collected fry in large outdoor concrete tanks. Several males produced mature sperm at age 5 years (the normal period to sexual maturity in Taiwan is 4–5 years), and a single 6-year-old female was found to contain oocytes in the yolk vesicle stage. The authors suggest that a few more months rearing may be sufficient to achieve the critical egg diameter at which induction of ovulation may be attempted. This limit has tentatively been set at 0.7–0.8 mm by Nash and Kuo (1976), who also provide information concerning the husbandry of captive broodstock.

Although success in the artificial spawning of milkfish has been had with only a few fish, the results are sufficiently encouraging to suggest that control over reproduction will be gained within the next few years. Nash and Kuo (1976) and Vanstone et al. (1976b) reported ovulation in captured females injected with partially purified salmon gonadotropin (SG-G100); fertilization was not achieved in these preliminary experiments. The most significant results to date have come from researchers at the Southeast Asian Fisheries Development Centre in the Philippines, who succeeded in inducing ovulation in two freshly caught mature female milkfish injected with a mixture of salmon pituitary acetone powder and human chorionic gonadotropin (HCG). The eggs were fertilized with milt obtained surgically from naturally spawned males containing traces of viable milt and were incubated in a 4-m diameter rubberized canvas tank filled with filtered, aerated seawater to a depth of 1 m. Thirty-two larvae attained an age of 74 days on a diet of *Chlorella*, immature *Brachionus*, mixed wild zooplankton, and cultured mixed diatoms, at which time they were transferred to larger tanks with natural algal blooms and fed a supplemental diet of minced fish (Vanstone et al. 1977). As this review goes to press, the 32 fish are still alive (W.E. Vanstone, personal communication). Both HCG and salmon pituitary acetone powder are relatively inexpensive, and it is encouraging to note that ovulation may be induced without the use of the more costly SG-G100.

CATFISH

Catfish are a high quality food fish with con-

(Table 2 con't.)

ΔT^a (h)	Delay to ovulation (h)	Fertilization method	Incubation method	Results	Notes	Reference
–	8–12	Stripped; dry	Fertilized eggs spread on weeds in *hapa*	No results given	Female may be catheterized to determine degree of egg ripeness. Injection of females with HCG increases the number responding: 1st injection: 100–250 IU added to pituitary extract; 2nd injection: 300–700 IU	Potaros and Sitasit (1976)
6	4	Stripped; dry	Fertilized eggs attached to aquatic weeds and spread in hatching containers	Ovulation with 100% fertility	–	Boonbrahm et al. (1968)
8–10	18–24	F: stripped M: testes removed and strips mixed with eggs	Nylon screen in hatching pond	Fry survival 80–90%, 40 days after hatching	Small, young males injected with half female dosage at time of second female injection	Chen (1976)
–	–	Natural spawning	–	Average spawning response of 58% obtained using HCG, hatching rate 68%	Female may be catheterized with medicine dropper for ovarian biopsy. 75% spawning obtainable using catfish pituitary extract but cost greater than HCG	Carreon et al. (1976)
–	–	Natural spawning in small paddy fields (3.5 × 3 m; 15–20 cm deep)	–	Response close to 100%	Pituitaries of marine catfish *Tachysurus* also effective	Anonymous (1977 c)

siderable tolerance of crowding and adverse environmental conditions, and members of the genera *Pangasius* and *Clarias* are assuming increasing importance in aquaculture operations in Southeast Asia and India. Members of the family Clariidae are particularly hardy, for they possess an accessory air-breathing organ that enables them to survive in oxygen-poor waters. *Clarias batrachus* and *Clarias macrocephalus* are extensively cultivated in Thailand, where the availability of cheap food in the form of trash fish from the offshore fishing industry has stimulated production dramatically within the last decade.

Of those species of catfish cultivated in any numbers in Southeast Asia and India, only *C. batrachus* spawns in captivity, and recent years have seen the beginnings of an effort to develop techniques of induced spawning. The literature on this subject is, however, not extensive; the critical comment made earlier concerning the literature on induced spawning in carps is applicable to catfish. The incomplete nature of many of the reports is evident in Table 2. The explanatory notes on the induced breeding of carps are generally applicable. Brief mention should, however, be made of differences concerning the environmental control of reproduction and techniques of incubation.

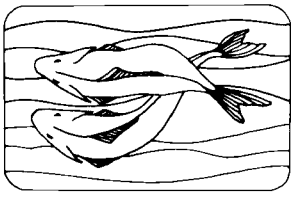
ENVIRONMENTAL CONTROL OF REPRODUCTION

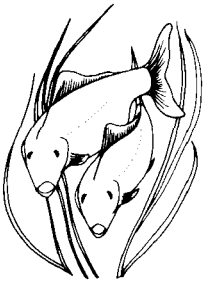
The spawning of Asian varieties of catfish is, like that of other pond-reared fish, seasonal; attempts to induce ovulation through hormonal injection are generally made between May and October. We have already noted that, in carp, it has often been possible to spawn the same female more than once in a single season. Although no such reports exist for catfish reared under field conditions, recent research on the Indian catfish *Heteropneustes fossilis* strongly suggests that this is feasible. Sundararaj and Vasal (1976) have shown that female catfish may become gravid 2 months earlier than in nature following exposure to a long photoperiod (14 hours/day) at a temperature of 25 °C for 6 weeks and that spawning may be induced successfully in these fish through administration of ovine luteinizing hormone. Furthermore, continued exposure of the spent

females to the same photoperiod regimen prompts reinitiation of vitellogenesis within 30 days, with the result that these fish could again be artificially induced to spawn. The process has been repeated successfully as many as four times in the period between April and July of the same year, thus providing four crops of eggs in the period usually taken to produce one. The importance of these findings for catfish culture is considerable, and it is probable that, with suitable refinement, the technique will be applicable to other cultivated species of fish. Further research is needed to ascertain whether similar environmental manipulation is possible under field conditions.

INCUBATION OF FERTILIZED EGGS

Commonly, fertilization of catfish eggs is allowed to proceed through natural spawning, although stripping and dry mixing are occasionally carried out. As has already been noted in the case of carp, the highest hatching rates are obtained when fertilized eggs are collected and incubated under controlled conditions; yet little sophistication is apparent in the methods currently employed for catfish in Asia. Catfish eggs are small and adhesive and may be spread on nylon screens submerged in small, stagnant hatching ponds provided with aeration (Chen 1976); more commonly they are allowed to attach to bundles of aquatic weeds or palm fibres, and these "egg carriers" are then floated in a hatching *hapa* (Potaros and Sitasit 1976; Boonbrahm et al. 1968). More elaborate arrangements involving hatching troughs suspended in running water have been devised for the large-scale catfish farming industry in the southern United States (Bardach et al. 1972), and it is probable that yields of fry in Asia could be considerably increased through the adoption of similar techniques.





5. METHODS OF OVARIAN BIOPSY

The amount of hormone required to bring about ovulation varies directly with the state of maturity of the oocytes, and it is likely that lack of success in many hypophysation attempts may be traced to inadequate assessment of egg development. It has already been noted that such external characteristics as "swollen belly" and "pinkish cloaca" are crude and unreliable indicators of ovarian development and should be replaced as soon as possible by more accurate methods. A suitable method would satisfy the following criteria: performance must be rapid and technically simple, elaborate equipment should not be required, and the procedure should be minimally stressing to the fish so that it may be carried out repeatedly if necessary.

The most promising approach at present appears to be the removal and examination of eggs. Removal is generally accomplished through the process of catheterization, in which a fine plastic tube (the diameter is varied according to the size of the eggs) is passed into the ovary by way of the genital pore, and a small sample of eggs removed by suction. This method works well for carp, mullet, and catfish, although care must be taken to ensure that rough handling of the fish does not result in atresia (Chen et al. 1969). The use of anesthetics should be investigated in this regard. A method involving abdominal puncture has recently been reported by Bienarz and coworkers (1977) and has proved safe for repeated sampling in the common carp *Cyprinus carpio*. In this technique, a needle of 2.5 mm diameter is used to pierce the abdominal wall 2 cm above the abdominal fin, and samples are withdrawn from a depth of 2–3 cm.

Information concerning the state of maturity of the eggs is provided by colour, histological appearance, and size. Colour of eggs is commonly employed as a criterion of maturity in carp; yet interpretation is clearly subjective, and published reports are often contradictory. More information may be obtained through histological examination of the oocytes: the microscopic appearance of eggs in various stages of development has been described for carp (Chen et al. 1969; Bienarz and Epler 1977) and for mullet (Kuo et al. 1974). Histological procedures are, however, time consuming and involve subjective interpretation of the results, and it is likely that the quickest and most reliable method of biopsy will rely on oocyte size alone.

A biopsy based on the principle of mean oocyte diameter has been well worked out for the mullet *Mugil cephalus* by Shehadeh et al. (1973b). Several oocytes are removed by suction from an unanesthetized female through a polyethylene cannula inserted into the oviduct to a distance of 6–7 cm from the genital pore. The eggs are washed, fixed in 1% formalin in 0.6% NaCl, and placed on a Plexiglas plate for measurement with an ocular micrometer. The effective dose of hormone is inversely proportional to mean egg diameter and best results are obtained when egg diameter is greater than 0.65 mm. A culturist can estimate dosage simply by interpolating on a graph relating mean egg diameter to effective dose (Fig. 8). The method is accurate, quickly performed, and, if carefully carried out, safe for repeated samplings.

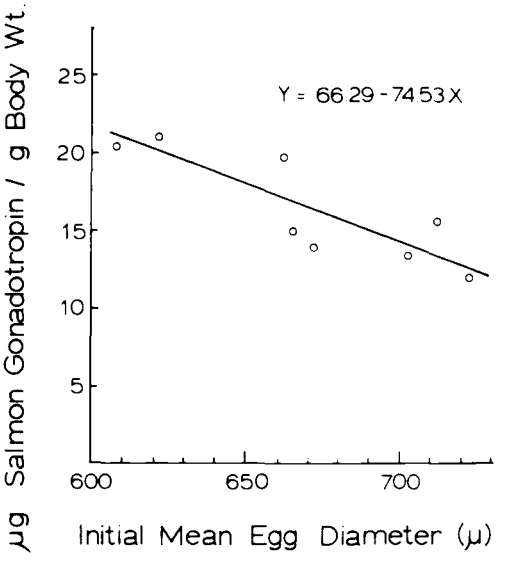
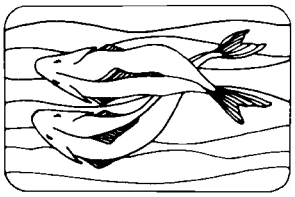


Fig. 8. *The relationship between initial mean egg diameter of recipient female mullet and the amount of gonadotropin required to induce spawning. After Kuo et al. (1974).*

Its adaptation to other species of fish can, however, only be successful if:

- The anatomy of the female genital tract is such that the path taken by the cannula is predictable. In mullet, carp, and catfish there appears to be little problem in this regard, for the ovary and the oviduct are continuous, and a tube introduced into the genital pore will invariably pass into the ovarian mass. In milkfish, however, ripe eggs are shed into the peritoneal cavity and pass from there to a collecting funnel leading to the genital pore. In this case the ovary and oviduct are not continuous, and a cannula passed through the genital pore may pierce internal organs. Use of this technique may thus be inappropriate in this species (Nash and Kuo 1976).
- Oocytes develop in synchrony; that is, there should be no significant difference in the state of maturity of samples of oocytes taken from different regions of the ovary. The method will, for example, not be reliable if eggs removed from the anterior portion of the ovary are consistently more advanced in maturity than those removed from the posterior segment, for exact placement of the cannula cannot always be achieved. Synchronous development has been demonstrated in the mullet (Shehadeh et al. 1973b), and studies should be undertaken to ascertain whether this is the case in other cultured species of fish.
- Diameters of oocytes must be known for all stages of maturity, so that an "atlas" of egg development may be assembled for each species. This information is available for mullet and at present is being gathered for milkfish (Nash and Kuo 1976); similar studies on carp and catfish should be undertaken to determine whether mean diameter may reliably be used as an indicator of oocyte maturity.



6. PRESERVATION OF GAMETES

Preserving viable gametes of cultured fish is desirable as a means of making good any deficiencies of supply, as a means of enabling breeding to occur whether or not maturation of males and females coincides, and as a means of establishing a reserve of genetic material of known quality for the initiation of programs of selective breeding. These objectives are in general realized through the storage of sperm, and this problem has received the most attention. The storage of ova of cultured species is unlikely to assume great importance except in instances where gametes of a desirable genetic makeup are to be conserved as a means of extending the breeding base. Work in this area is still in the experimental stage, and only passing mention will be made here.

In the groups of fish considered in this review, eggs and spermatozoa are shed simultaneously into the surrounding water, with appropriate reproductive behaviour ensuring correct timing and positioning of the male and female so that immediate mixing and fertilization may take place. Milt is defined as a suspension of individual spermatozoa in the seminal fluid resulting from hydration of the testes. It is important to note that spermatozoa do not become motile until contact with water is made. The process of acquiring motility is termed activation and occurs the instant milt is liberated into the surrounding water. Water, in this context, refers to the medium in which fertilization normally occurs and may thus be freshwater, brackish water, or seawater, depending on the species. The stimuli responsible for sperm activation in most species of cultured fish have not been identified; pH, osmotic pressure, and ionic content of the surrounding medium may all be important (Hines and Yashouv 1971).

The motility of spermatozoa following activation is variable. It is much longer-lasting in fishes spawning in brackish water and in seawater than in those spawning in freshwater; an extreme case is the Pacific herring *Clupea harengus*, in which spermatozoa may remain motile for 4–5 days (Yamagimachi 1957). In most fishes spawning in freshwater, however, spermatozoa remain motile for no longer than 2–3 minutes. The duration of energetic movement in the common carp *Cyprinus carpio*, for example, is between 30 and 60 seconds, and detectable motility is absent after 5 minutes (Ginzburg 1972). Although motility is not in itself an absolute guarantee of fertility, spermatozoa that have become immotile are no longer capable of fertilization. As a general rule, then, it is essential that contact of stored milt with water be avoided until just prior to mixing with eggs.

PRESERVATION OF SPERM

Sperm may be preserved for either a short or a long term. The former may be undertaken for reasons of convenience: spermiation may be induced in advance of ovulation, with the result that milt is on hand when the female is stripped, and only one fish need be handled to effect fertilization. It is a relatively simple procedure, involving maintenance of fresh semen on ice or in a refrigerator at temperatures between 0 and 10 °C. The period of storage may range from a few hours to 5 days. Long-term storage (cryopreservation) is carried out at much lower temperatures (between −20 and −196 °C) and should ideally maintain viable sperm for periods of several years. Most techniques of cryopreservation presently in use involve rapid cooling and storage in either solid CO_2 or liquid nitrogen (Liq.N_2). Recent results with vacuum-dried salmonid milt are encouraging but preliminary (Zell 1978).

Although considerable research has been carried out on sperm preservation in salmonids (see Wiltzius 1973; Horton and Ott 1976), comparatively little attention has been directed to tropical and subtropical cultured species. The few studies on the preservation of carp sperm are preliminary, and to date only two reports of the cryopreservation of mullet sperm have been published. As a result of this paucity of information, the following discussion is general and attempts to present certain principles that, though worked out largely for salmonids, are likely adaptable to other fish. Details of techniques that have proved at least partially successful with carp and mullet (none have appeared for milkfish and catfish) are presented in Table 3; the wide variation in procedure

Table 3. Cryopreservation of carp and mullet sperm.

Fish (reference)	Diluent	Cryo-protectant	Ratio	Equili-bration	Freezing rate	Storage	Thawing technique	Success
Cyprinus carpio (Moczarski 1977)	Alsever's solution[a]	DMSO 10%	1:1/ 1:2	5 sec	Ampoules held 3–5 cm above Liq.N_2 surface until frozen	Liq.N_2; –196 °C; duration not stated	30 °C water bath	12% fry achieved; 47% fertilization
Cyprinus carpio (Stein and Bayrle 1978)	Mixture[b]	DMSO 10%	1:3	0 sec	0.2 ml pellets on solid CO_2	Liq.N_2; –196 °C; 7 days	3 pellets/10 ml 1% $NaHCO_3$; shaken	High motility; no fertilization
Cyprinus carpio (Pavlovici and Vlad 1976)	Mixture[c]	DMSO 5%	1:3	60–180 min; 0–2 °C	Ampoules in Liq.N_2 vapours; rate not stated	Liq.N_2; –196 °C; 24 h	21 °C water bath; 1–2 min	11% fertilization
Aristichthys nobilis (Sin 1974)	Mixture[d]	Combination: DMSO 7.4%/ glycerine 9.3%	1:9[e]	–	CO_2/acetone; rate not controlled	CO_2; –79 °C; 30–60 min	6–10 °C water bath	2.7%–5.7% fertilization
Cyprinus carpio, Aristichthys nobilis, Ctenopharyngodon idellus (Anonymous 1974)	–	Ethylene glycol	–	–	–	Liq.N_2; –196 °C; 1 y	–	Produced 1/10 as many larvae as controls
Cyprinus carpio, Labeo rohita, Puntius gonionotus (F.C. Withler, unpublished)	Mixture[f]	DMSO 10%	1:4	0 sec	Ampoules held 2 cm above Liq.N_2 surface 5–10 min	Liq.N_2; –196 °C; 24 h	25–30 °C water bath	*C. Carpio* and *P. gonionotus*: 5–20% motility; *L. Rohita*: 58% survival to early fry stage
Mugil cephalus (Pruginin and Cirlin 1976)	51% sea-water; 34% distilled water	Glycerol 15%	1:1	–	CO_2 pellets; 1 min	Liq.N_2; –196 °C; 2–4 months	–	"Some" motility; no fertilization
Mugil cephalus (Chao et al. 1975)	Marine teleost Ringer[g]	DMSO 10%	1:1	≤1 h	0.5 ml straws held in Liq.N_2 vapour 4 min	Liq.N_2; –196 °C; 1 y	"Room temperature" water bath; 4–5 min	2.7% fertilization; 31.85% hatching[h]

[a]Alsever's solution (Hodgins and Ridgeway 1964: sodium citrate ($C_6H_5Na_3O_7$), 0.8%; dextrose, 2.05%; NaCl, 0.4%.

[b]NaCl, 750 mg; $NaHCO_3$, 200 mg; Na_2HPO_4, 53 mg; $MgSO_4 \cdot 7H_2O$, 23 mg;KCl, 38 mg; $CaCl_2 \cdot 2H_2O$, 46 mg; glucose, 100 mg; glycine, 500 mg; egg yolk, 20 ml; H_2O, 100 ml.

[c]KCl, 0.75%; lecithin, 10%; H_2O, up to 100 ml.

[d]NaCl, 0.6%; KCl, 0.038%; $CaCl_2 \cdot 2H_2O$, 0.023%; $HaHCO_3$, 0.1%; $NaHPO_4 \cdot H_2O$, 0.041%; $MgSO_4 \cdot 7H_2O$, 0.023%.

[e]Motility was greatly increased at a dilution of 1:3; yet sperm at this dilution was unavailable for fertilization trials.

[f]NaCl, 730 mg; $NaHCO_3$, 500 mg; fructose, 500 mg; lecithin, 750 mg; mannitol, 500 mg; H_2O, 100 ml.

[g]Marine teleost Ringer solution (Burton 1975): NaCl, 231 mM; KCl, 8 mM; $CaCl_2$, 2.2 mM; $MgCl_2$, 3.7 mM.

[h]When 10% glycerine was added as cryoprotectant, the following results were obtained: 2.47% fertilization, 52.5% hatching.

is evidence of the early stage of development of this field. It should be noted that cryopreservation of sperm is carried out routinely at artificial insemination facilities for livestock and that liquid nitrogen is generally available at these institutions. Liaison between fish breeders and livestock breeders should thus be encouraged.

CRYOPRESERVATION OF SPERM

Collection of milt: Culturists must at all costs avoid activating spermatozoa and must, therefore, keep both genital pore and collecting glassware scrupulously dry. To this end it is advantageous to anesthetize the donor and to withhold food for a day before stripping to ensure voiding of the digestive tract.

It is often necessary, particularly under field conditions, to store freshly collected milt for several hours before dilution and freezing is carried out. Experience with short-term preservation suggests that this should be done on ice and that the jars should be sufficiently large to permit adequate gaseous exchange. Horton and Ott (1976) suggest a 1:10 ratio of semen to air space if the jars are sealed.

Preparation and addition of diluent: A suitable diluent (also referred to as an extender) must maintain sperm alive but inactive prior to freezing; it should be isotonic with semen and should be buffered to counteract the acidity or alkalinity of the cryoprotective agent (F.C. Withler, personal communication). Innumerable variants have been tried, most of them based on physiologic saline for marine or freshwater teleosts, with or without the addition of various "nutrients" and "stabilizers" (e.g., fructose, mannitol, egg yolk, lecithin, glycine). Horton and Ott (1976) recommend that the number of constituents be kept to a minimum and that the concentration of each be determined experimentally on the basis of observed sperm motility. This simple approach appears to be the correct one. Of the reported attempts to cryopreserve carp and mullet sperm summarized in Table 3, the most successful has been one in which an unmodified marine teleost Ringer solution was employed as diluent (Chao et al. 1975). An empirical approach is, however, unavoidable, for success in transferring diluents from one fish to another is completely unpredictable: Ott (1975) notes that different diluents and concentrations of cryoprotectant were necessary for each species of *Oncorhynchus*; yet several diluents used routinely for freezing salmonid sperm gave encouraging preliminary results with the common carp *Cyprinus carpio* and the Indian carp *Labeo rohita* (F.C. Withler, unpublished).

There is little agreement concerning the amount of diluent to be added, and this, too, must be determined experimentally. Successful sperm: diluent ratios for salmonids range between 1:4 and 1:9 (Ott 1975; Horton and Ott 1976). However, Moczarski (1977) found the viability of cryopreserved common carp sperm to be greater at 1:1 or 1:2 than at 1:6. Sin (1974) noted a similar effect with silver and bighead carp, in which motility and viability were greatly increased as the dilution ratio decreased from 1:9 to 1:3. Successful long-term cryopreservation of mullet sperm has been achieved using a dilution ratio of 1:1 (Chao et al. 1975). Whatever the ratio, diluent and sperm should be isothermal before mixing.

Cryoprotectant: Freeze-thaw damage is minimized by the addition of such agents as DMSO (dimethyl sulfoxide), ethylene glycol, or glycerol to the diluent before mixing with sperm. DMSO is recommended for salmonids (Horton and Ott 1976), and the greatest success with cryopreservation in cultured fish has been obtained using this protectant (Table 3). The concentration employed in the diluent must be determined experimentally; 10% is commonly used. The rapidity with which DMSO penetrates cellular membranes appears to make any equilibration between sperm and extender unnecessary, and freezing should begin at once. Chao and coworkers (1975) allow equilibration for up to 1 hour before freezing mullet sperm yet provide no justification for this practice.

Rate of freezing: The majority of cryopreservation techniques involve storage of frozen sperm in Liq.N_2 at −196 °C; this may be done in 1 ml aliquots in glass ampoules or in 0.5 ml plastic straws sealed at both ends. Initial lowering of the temperature of the sample may be achieved either by suspending the diluted and protected sperm in Liq.N_2 vapour or, less commonly, by forming pellets on solid CO_2. The former technique allows control of the rate of freezing, with fastest freezing taking place in vials closest to the surface of the liquid. Freezing usually takes 4–5 minutes at a distance of 2 cm from the surface. Much faster rates of cooling are obtained with the CO_2–pellet technique (Nagase 1964), in which aliquots of diluted semen are dropped into holes in solid CO_2 for about 1 minute, then removed for storage in covered test tubes in Liq.N_2. This technique has been employed for common carp (Stein and Bayrle 1978) and mullet (Pruginin and Cirlin 1976). There is little agreement concerning the optimum time required for freezing of teleost sperm: the process must occur rapidly enough that thermal shock is minimal yet not so fast as to allow the formation of intracellular ice crystals (Meryman 1966; Ferrant 1972).

Thawing and fertilization: Horton and Ott (1976) recommend that cryopreserved salmonid sperm be thawed as rapidly as possible and that this be achieved by swirling the ampoule in water at 50–60 °C. Withler and Morley (1968), however, obtained equally good results with salmonid sperm thawed at 11 °C and at 45 °C; the effect of this factor has not been systematically studied in carp and mullet, and as has been shown in Table 3 a variety of methods has been tried. Sperm frozen by the CO_2–pellet technique must be thawed by the addition of 1% $NaHCO_3$ (Stein and Bayrle 1978). Because the motility of thawed sperm, once activated, lasts only a few seconds, the contents of the ampoules should be combined with fresh ova as soon as the slush stage is reached.

SHORT-TERM PRESERVATION OF SPERM

Lack of adequate facilities in many cases prohibits a program of cryogenic storage of milt. The technical demands of short-term storage are

more easily met, and this technique can greatly benefit culturists, because collection of semen even a few hours in advance of stripping the female is a simple way to streamline the mixing of eggs and sperm. In addition, culturists may ascertain the motility of a small aliquot before use and in this manner gain at least a rough idea of the overall viability of the sperm. Present methods of short-term storage are, however, poorly worked out and contradictory, and there is need for a coherent research effort to standardize procedures.

Confusion exists, for example, concerning the dilution of semen. The milt of rainbow trout remains fertile after storage at 4 °C under O_2 or air for 21 days in an undiluted state (Stoss et al. 1978), and F.C. Withler (unpublished) has shown that the undiluted semen of the common carp *Cyprinus carpio*, the catfish *Pangasius sutchi*, the Indian carp *Labeo rohita*, and the tawes *Puntius gonionotus* can be activated up to 24 hours after extrusion if kept chilled in a refrigerator or on ice. Similarly, the undiluted milt of the grey mullet *Mugil capito*, stored at 10 °C, could be activated by the addition of seawater within 36 hours; the number of swimming spermatozoa is, however, low (Hines and Yashouv 1971). Various diluents have, nonetheless, been tried. Mullet sperm, when diluted 1:1 with marine teleost Ringer solution (Burton 1975) and stored at 5 °C, maintained motility for 23 days, and fertilizing capacity after 3–6 days storage was 1–3% (Chao et al. 1975). Ringer solution was found to be superior to seawater for prolonging motility after activation. J.D. Funk (unpublished) has carried out preliminary sperm preservation trials with *Puntius gonionotis* in Malaysia and notes that semen diluted 4:1 with Cortland saline (Wolf 1963) produced 74% fertilization after 5 days storage at 1–5 °C. Milt from the Indian carp *Labeo rohita* and the common carp *Cyprinus carpio* showed motility following storage for 72 hours at 0–5 °C in a diluent of 1% glycerine in frog Ringer solution (Wolf 1963); elimination of the glycerine had a deleterious effect on sperm survival (Bhowmick and Bagchi 1971). Milt from *L. rohita*, when stored in Ringer-glycerine for 4 hours at 28 °C, maintained its fertilizing capacity.

It is not yet clear whether use of a diluent is advantageous or whether it represents an extra step of dubious value. Opinion is divided as to the inclusion of cryoprotectants; at temperatures higher than 0 °C these do not appear to be necessary. Any diluent must maintain spermatozoa alive but inactive; addition of water to carp sperm, for example, causes immediate activation lasting less than a minute, and the stripping of milt for storage must, therefore, be performed under dry conditions. Because the stimuli responsible for sperm activation in most species of cultured fish have not been identified, the composition of a diluent must be arrived at empirically. Once this is accomplished, however, the most appropriate moment for its addition to milt remains open to question, for dilution need not occur prior to refrigeration. Milt may be diluted *after* storage at low temperatures and allowed to incubate in the diluent for up to 1 hour before addition to fresh ova. Zell (1978) reports that the percentage of eyed eggs produced by undiluted rainbow trout milt stored for 2 days at 0 °C increased from an average of 10 to 78 following incubation in Poulik's solution. This "restoration" of aging sperm has also been noted by Funk (unpublished) in Chinese carp.

Provision must be made for gas exchange in refrigerated milt. Vials should not be more than three-fourths filled and must be left unsealed. Stirring should be avoided (Stoss et al. 1978).

CRYOPRESERVATION OF OVA

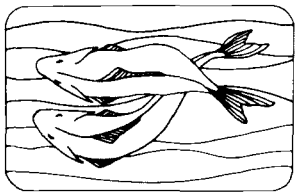
The size and complexity of teleost ova make their cryopreservation a technical challenge far greater than that presented by spermatozoa; however, the successful cryopreservation of mammalian embryos suggests that the difficulties are not insurmountable (Whittingham et al. 1972; Leibo 1977). Zell (1978) reports successful cryopreservation of unfertilized teleost eggs and zygotes. In studies with Atlantic salmon, rainbow trout, and brook trout ova, unfertilized eggs frozen to −20 °C in liquid nitrogen for 5 minutes were fertile after thawing, and high percentages of zygotes or eyed eggs survived exposure to temperatures as low as −50 °C. The freezing medium was Hanks' salt solution (Wolf 1963); no cryoprotectants were added. Hatching of all eggs supercooled or frozen to −12 or −5 °C was uniformly high; control over the rate of cooling was determined to be the major technical difficulty.

CRITERIA FOR SUCCESS

From Table 3 it is clear not only that few studies have been carried out on cryopreservation of sperm of cultured species of fish but also that, in those that have, criteria for the degree of success are, to say the least, disparate. To describe sperm as "highly motile" is less than rigorous; it is not clear whether this means that a large proportion of those sperm observed exhibited some sort of

motility, or whether an exceptional vigour on the part of individual sperm cells was observed. Fertility is a better yardstick; yet this, too, carries little meaning unless an attained stage of development is specified. Hatching represents, however, an unambiguous event; it occurs sufficiently late in development to represent a true viability of gametes yet not so late that an experiment in cryopreservation becomes an experiment in larval rearing. It is suggested that this criterion be adopted as the measure of success in future studies.

Few published reports of sperm preservation experiments note the sperm:ova ratio employed in subsequent fertility tests. This factor is, however, of critical importance: the application of 2-ml thawed sperm to a half-dozen eggs will, for example, almost certainly produce an unrealistically high rate of fertilization. It is therefore further suggested that the approximate number of sperm and ova used for each fertilization experiment be reported.



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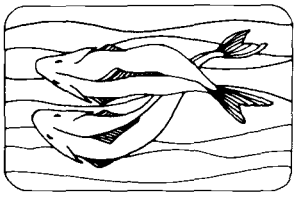

SUPPLIERS OF SPAWNING MATERIALS

Syndel Laboratories Ltd, 8879 Selkirk Street, Vancouver, BC.

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