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Effects of stress on fish reproduction, gamete quality, and progeny ☆

Carl B. Schreck *, Wilfrido Contreras-Sanchez, Martin S. Fitzpatrick

Oregon Cooperative Fish and Wildlife Research Unit,[1] U.S.G.S. and Department of Fisheries and Wildlife, 104 Nash Hall, Oregon State University, Corvallis, OR 97331-3803, USA

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Abstract

Different taxa of fish have different tolerances to stress. This implies that for a particular stressor, severity may vary depending on the species to which it was applied. Species may differ in the nature of their physiological response and reproductive consequences to stressors. For example, disturbance or handling may affect the timing of reproduction—accelerating or delaying it as the case may be—in species such as rainbow trout (*Oncorhynchus mykiss*); however, tilapia (*Oreochromis niloticus*) respond by acceleration or complete inhibition of reproduction, depending on the maturational stage when the stressor is experienced. Strategies for coping with stress affect reproductive fitness either in terms of gamete or progeny quality. The physiology associated with maturation and spawning appears tightly coupled with stress physiology. Environmental variables, particularly nutrition, are ultimately important in affecting gamete quality and reproductive timing. The physiological response to stressors is also quite polymorphic, within and between species. For example, the circulating concentration of the primary stress response factor cortisol varies greatly among resting and among stressed rainbow trout stocks. Immunocapacity can be influenced by stress, reducing reproductive fitness of broodfish. We propose that maternal systems have been developed to buffer eggs from deleterious consequences of stressors, including regulation of transfer of substances of maternal origin to the egg and in mechanisms controlling the timing of reproduction. Effects of nutritional stressors are moderated by effects on timing of first maturity or subsequent reproductive events and/or by maintenance of quality of some eggs

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* Corresponding author.

E-mail address: carl.schreck@orst.edu (C.B. Schreck).

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via atresia of others. Deleterious overload of eggs with substances such as cortisol is likely prevented by limiting entry of these compounds into the eggs. Barriers to vertical transmission of numerous pathogens seem to exist, while maternally derived immune protection is provided to assist with disease prevention of pathogenic organisms acquired from parents or by direct post-spawning infection. Timing of reproductive events including puberty, atresia, maturation and ovulation are influenced by other physiological variables responsive to stressors. Knowledge of how a stressor might affect the physiology of a species can help in development of management tactics that lessen the impact of a stressor or even in the development of therapeutants. Published by Elsevier Science B.V.

Keywords: Stress; Fish reproduction; Gamete quality; Progeny

1. Introduction

Adult fish are exposed to stressful situations in the wild as well as under culture conditions. The nature of the physiological responses to threats seems stereotypic at a gross level amongst life history stages once fish have begun feeding exogenously. However, the stress response can be polymorphic with regard to species of fish, stage of maturity, and type and severity of stressor (for general discussion of these topics in fish, see Donaldson, 1981; Schreck, 1981, 2000; Barton and Iwama, 1991; Wendelaar-Bonga, 1997; and for discussion of stress and reproduction, see Pankhurst and Van Der Kraak, 1997). The word stress has been defined in several ways (Pickering, 1981). Here, a physiological nomenclature is used referring to *stress* as the response of the body, i.e., a physiological cascade of events that occurs when the organism is attempting to resist death or reestablish homeostatic norms in the face of an insult. It is our aim in this paper to describe the stress response of adult fish and how it relates to reproductive fitness.

Comprehending the population-level consequences of stressful events requires understanding the effects of stress on an individual; such understanding is critical for conservation biology, stewardship of wild populations, and aquaculture. From an ecological as well as a management perspective, factors affecting broodfish quality can be reflected in the number and quality of their progeny. Stressful situations can overwhelm the homeostatic mechanisms of a fish, thereby placing a load on the body in an attempt to compensate and achieve another level of stasis. When stressed, fish can assume a different mode of operating, *allostasis* (see Sterling and Eyer (1988) and McEwen (1998) for discussions of the concept), that is adaptive in terms of keeping the animal alive in the face of the stressor but can be maladaptive in terms of performing other necessary life functions or of reproductive fitness (Schreck, 2000). This paper briefly describes the basic physiological response to stressors and consequences on fitness of broodfish. We then review effects of stressors applied at different times during reproductive maturation and speculate about effects of stressor severity. The polymorphic nature of the stress response is exemplified by comparison of the stress-invoked concentrations of one of the primary stress factors, cortisol, among species and among stocks of a rather well-studied organism, the rainbow trout, *Oncorhynchus mykiss*. The immune system is then discussed in some depth since there is little or no information

concerning other physiological functions of this steroid. It is not surprising that the response of broodfish to stressors in terms of physiology or reproductive tactics is extremely varied given the great diversity in reproductive modes found amongst fishes, with the extant forms divisible into 32 reproductive guilds (Balon, 1975). To help understand effects of life history phenotypes, the responses by fish with very different life histories are compared. In conclusion, a model that describes the interaction of maternal systems with eggs to provide protection of eggs from maternal stress-induced responses is proposed.

2. Effects of stress on adult physiology and quality

2.1. The physiological stress response

Most stressful situations induce a rather predictable stress response in fish similar to the General Adaptation Syndrome (Selye, 1950, 1973). The stress response begins as changes in tissue and organ function that attempt to cope with or compensate for the stressor, resulting in a move away from homeostasis—these changes are the physiological response that may differ between individuals in rate or magnitude, but share general characteristics in their mode and action. Since the elements of the physiological stress response have been described elsewhere (Donaldson, 1981; Schreck 1981, in press; Barton and Iwama, 1991; Wendelaar-Bonga, 1997), only a brief overview will be presented here, recognizing that much of our understanding of how fish respond to stressors is based on the study of juvenile life history stages. The physiological stress response happens following perception of the stressful event. Most stressors induce a neuroendocrine cascade involving an immediate release of catecholamines and activation of the hypothalamic–pituitary–interrenal axis (HPI). Complete activation of the HPI lags somewhat behind the catecholamine response because of the time needed for neural stimulation of release of the neuropeptide corticotropin releasing factor (CRF) originating from the hypothalamus that causes pituitary synthesis and secretion of corticotropic hormone (CTH), which must travel via the circulation to the interrenal to stimulate synthesis and secretion of glucocorticoid hormones (cortisol in teleosts). Together, catecholamines and glucocorticoids initiate secondary and tertiary stress response factors. The physiology of the relationships between stress and reproduction for the poikilothermic vertebrates was reviewed by Greenberg and Wingfield (1987).

One aspect of the secondary responses consists of mobilization of energy-rich substrates by depletion of hepatic glycogen stores, elevation of plasma levels of glucose, effects on circulating levels of free fatty acids, and general inhibition of protein synthesis (see Mazeaud et al., 1977; Pickering, 1981). In this regard, stress can be viewed as being anti-anabolic (Schreck, 1992). Stress is also manifested in hydromineral balance, resulting in water loading in fish in fresh water and loss in those in salt water. Electrolyte concentrations can also be affected, but it is unclear if these are direct responses to a primary stress factor or are more indirect due to water flux. Osmoregulatory consequences produce an allostatic load. Stress also encompasses various arms of the immune system; stress is generally believed to result in depressed immune capacity.

There are numerous tertiary responses resultant for the primary and secondary stress responses. For example, normal behavior can be impaired (Schreck et al., 1997).

Of course, a particular stressor can also cause a specific effect or effects in fish that are the direct result of that stressor alone. Toxicants serve as good examples of stressors that have specific actions because they target specific physiological systems. In fact, certain toxicants may not activate the more general stress response system at all. Because of the highly variable effects of contaminants on the physiology of fish, this category of stressor will not be considered further in this review.

Taken together, the allostatic load due to stress appears to reduce reproductive fitness in fish (Schreck, 2000). This can be manifested directly in reduced survivorship of the adult or via reproductive failure. Reproductive impacts can be attributable to effects on maturation, time of spawning (or ovulation), gamete quality, and progeny quality. These factors will be discussed in detail subsequently.

3. Variation in the physiological and organismic stress response

Numerous factors can contribute to the variation expressed in the physiological and organismal response to stressors. These include gender, age, season, physical condition, social status, water quality, and the nature of the stressor (Schreck, 1981; Wendelaar-Bonga, 1997). The inherited performance capacity and the stress-invoked phenotype can have enormous repercussions on the way a particular fish responds to stressors (Schreck, 1981; Pottinger et al., 1992; Pottinger and Moran, 1993).

3.1. Variation in the physiological stress response

3.1.1. Primary stress response factors

As mentioned above, the physiological response to stressors can be polymorphic with considerable variation between species. Thorough integrative and comparative studies have not been undertaken to contrast how inter- and intraspecific variation are manifested in the stress response; however, there is sufficient literature to suggest that different species can respond differently to similar stressors. For example, while salmonids respond to handling and crowding stressors with an almost immediate (i.e., measurable in minutes after the onset of the stressor) elevation in circulating levels of cortisol (Donaldson, 1981; Barton and Iwama, 1991), measurable increases in cortisol levels of the sea raven, *Hemitripterus americanus*, are apparent only an hour after the onset of the stressor (Vijayan and Moon, 1994).

Although fish, like other vertebrates, respond to stressors by changing physiological function to reallocate energy for the purposes of coping, the actual control and nature of the physiological response shows broad phylogenetic differences. For example, while cortisol is the predominant stress steroid of teleosts, our laboratory has been unable to document the presence of cortisol or any other glucocorticoid in adult Pacific lamprey, *Lampetra tridentata*, even following severe handling and prolonged crowding that resulted in elevation of glucose; also, no glucocorticoid was detected in this species when injected with the radio-labeled corticosteroid precursor pregnenolone (Close,

unpublished data). Variation among strains of the same species is also evident; e.g., the cortisol stress response has been shown to vary among stocks of rainbow trout (Pottinger et al., 1992; Fevolden et al., 1993; Pottinger and Moran, 1993; Pottinger and Carrick, 1999). Individual heritability for cortisol's elevation in response to stress is moderate to high (Pottinger and Carrick, 1999).

To understand the magnitude of the physiological variation in the physiological stress response extant within a species of adults, information on cortisol concentrations in the circulation from research on rainbow trout has been compiled (Table 1). This species and this physiological variable were selected because they were the most represented in the literature. Mean resting levels of cortisol varied from < 2.0 to 540 ng/ml, and the values after stress ranged from 8 to 735 ng/ml. Some authors have detected significant differences between males and females of the same age and strain (Campbell et al., 1994; Clements, 1996; Pottinger and Carrick, 1999). In this laboratory, the overall pattern of the cortisol response to acute stressors was found to change dramatically during the period when females were undergoing vitellogenesis (Contreras-Sanchez, 1995). These trout not only had higher than expected resting cortisol levels (mean = 51.5 ng/ml), but also exhibited a very rapid, but short duration elevation in plasma cortisol levels, peaking in 20 min and returning to pre-stressor concentrations within another 20 min. Such a rapid, attenuated response may be adaptive for gamete quality (see below). Of course, apparent differences could also arise from inconsistencies among methods.

Steroidogenesis was suppressed when ovarian follicles of rainbow trout were exposed in vitro to physiological levels of cortisol, suggesting a possible mechanism by which stress may compromise vitellogenic processes (Carragher and Sumpter, 1990). However, these results could not be reproduced by Pankhurst et al. (1995) and Pankhurst (1998). Wendelaar-Bonga (1997) presented an excellent review of related results, pointing out the difficulty in drawing conclusions because of inconsistencies in the data; negative effects of stress on reproduction mediated by cortisol were reviewed by Greenberg and Wingfield (1987).

The physiological response of a female to stress could have considerable consequence in terms of gamete quality and progeny fitness. Experiments in guinea pigs and mice indicate that the stress response in the mother, such as elevated levels of catecholamines and corticosteroids, can be reflected in the fetus and may cause alterations to the developing offspring (Dauprat et al., 1990; Takahashi et al., 1998). Several studies have shown the presence of large reservoirs of maternally contributed hormones in mature teleostean oocytes, supporting the idea that hormones could play a major role in regulating developmental processes post-fertilization (Lam, 1985; Brown et al., 1989; Brown and Bern, 1989; Feist et al., 1990; Schreck et al., 1991; Ayson and Lam, 1993; Yeoh et al., 1996a,b). Maternal transfer of hormones such as cortisol could be modified by physiological processes induced by stress, suggesting that concentrations in eggs could be affected (Campbell et al., 1992, 1994; Yeoh, 1993). Cortisol concentrations in coho salmon, *O. kisutch*, eggs were significantly higher when the fish were stressed for 2 weeks prior to spawning (Stratholt et al., 1997). McCormick (1998) also found that cortisol administered to ambon damselfish, *Pomacentrus ambionensis*, resulted in elevated ovarian cortisol concentrations, similar to those of fish experiencing stress in the natural environment. However, we were unable to find elevation in cortisol

Table 1
Resting and stressor-induced circulating cortisol levels in female, male and mixed sex populations of adult (post-puberty) rainbow trout of various stocks at various times during reproductive maturation

Gender and stage	Strain or location	Stressor	Duration of stress	Cortisol (mean ± S.E. or range controls at first sampling)	Cortisol after stress	Reference
Females, post-puberty	Sunndalsøra Hatchery	Confinement and Low water	30 min	n.a.	< 50–600 ng/ml ($n = 281$)	Fevolden et al., 1993
Mixed post-puberty	Annandale Strain Hatchery	Confinement	(a) 1 day (b) 7 days (c) 14 days	< 2.0 ng/ml ($n = 9$)	(a) 47.3 ng/ml ± 13 (b) ~15 ng/ml (c) 8.0 ng/ml ± 3.0	Pottinger et al.,
Females, post-puberty	n.a. Hatchery	Confinement	1 h	n.a.	144.3 ng/ml ± 15.9 ($n = 23$)	Pottinger et al., 1995
Females, post-puberty	Annandale Strain (low response) Hatchery	Emersion and Confinement	1 h	8.4 ng/ml ± 2.2 ($n = 13$)	172.8 ng/ml ± 20.0 ($n = 10$)	Pottinger et al., 1992
Females, post-puberty	Annandale Strain (high response) Hatchery	Emersion and Confinement	1 h	12.2 ng/ml ± 3.3 ($n = 11$)	404.1 ng/ml ± 28.2 ($n = 10$)	Pottinger et al., 1992
Mixed post-puberty	Rainbow Springs Hatchery	Cannulation and Chasing	5 min	~70 ng/ml ($n = 9$)	~170 ng/ml (immediately after) ($n = 9$)	Pagnotta et al., 1994
Mixed post-puberty	Linwood Acres Hatchery	Netting and Chasing	3 min	< 10 ng/ml ($n = 6$)	~80 ng/ml (1 h)	Vijayan et al., 1994
Mixed post-puberty	Apeldoorn Hatchery	Cannulation and Hypoxia	1.5 h	78 ng/ml ± 9 ($n = 5$)	735 ng/ml ± 424 (1.5 h after treat.) ($n = 5$)	Van Raaij et al., 1996
Females Maturing	n.a. Hatchery	Catheterization		ND ($n = 1$)	120 ng/ml (30 min) ($n = 1$)	Bry and Zohar, 1980
Females, maturing	n.a. Hatchery			7.7–10.5 ng/ml ($n = 41$)	n.a.	Bry, 1985

Females, maturing	Annan strain Hatchery	Confinement	2 weeks	7.2 ng/ml ± 1.5 ($n = 5$)	37.5 ng/ml ± 7.5 (after 2 weeks confinement)	Campbell et al., 1994
Females, maturing	Stirling strain (low response) Hatchery	Confinement	3 h	n.a.	56.4 ng/ml ± 3.4 ($n = 70$)	Pottinger and Carrick, 1999
Females, maturing	Stirling strain (high response) Hatchery	Confinement	3 h	n.a.	115.7 ng/ml ± 5.6 ($n = 75$)	Pottinger and Carrick, 1999
Females, maturing	Shasta strain Hatchery	Crowding, Netting, Draining, or Noise 45 days	1–5 min randomly daily	51.5 ng/ml ± 12.4 ($n = 10$)	128 ng/ml ± 29.2 (20 min)	Contreras-Sanchez, 1995
Females, maturing	Whitikau stream Wild	(a) Barrier	(a) 0–14 h	21.4 ng/ml ± 5.9 ($n = 9$–14)	(a) 144.7 ng/ml ± 22	Clements, 1996
		(b) Cage	(b) 1 h		(b) 549.1 ng/ml ± 60	
		(c) Crowding	(c) 1 h		(c) 432 ng/ml ± 33.0	
Females, maturing	Annan strain Hatchery	Emersion (complete drain)	3 min (for 9 months)	10.8 ng/ml ± 1.5 ($n = 10$)	33.8 ng/ml ± 5.9 (1 h; 4.5 months) ($n = 10$)	Campbell et al., 1992
Mixed Maturing	Sun Valley Hatchery	Hypophysectomy		540 ng/ml ± 35 ($n = 5$)	610 ng/ml ± 23 (immediately after) ($n = 5$)	Hill and Fromm, 1968[a]
Mixed–Maturing	Whitikau Stream Wild	Capture by Angling	(a) ≤ 5 min	(a) 34 ng/ml	(a) 85 ng/ml	Pankhurst and Dedual, 1994
			(b) 15 min	(b) 60 ng/ml ($n = 17$ and 15)	(b) 111 ng/ml (1 h; $n = 10$)	
Females, mid vitellogenesis	Russell Falls Hatchery	Confinement	4 h	14.9 ng/ml ± 5.0 ($n = 6$)	37.7 ng/ml ± 7.0 ($n = 12$)	Pankhurst, 1998
Females, Late vitellogenesis	Russell Falls Hatchery	Confinement	4 h	28.5 ng/ml ± 9.5 ($n = 12$)	145 ng/ml ± 29.6 ($n = 12$)	Pankhurst, 1998
Females, Ovulating	n.a. Hatchery			25.8–30.9 ng/ml ($n = 37$)	n.a.	Bry, 1985
Females, Ovulated	Shasta Strain Hatchery	Crowding, Netting, Draining, or Noise 45 days	1–5 min randomly daily	223.1 ng/ml ± 19.2 ($n = 20$)	257.2 ng/ml ± 30.1 (75 min; 45 days)	Contreras-Sanchez, 1995

(continued on next page)

Table 1 (*continued*)

Gender and stage	Strain or location	Stressor	Duration of stress	Cortisol (mean ± S.E. or range controls at first sampling)	Cortisol after stress	Reference
Males, maturation	Annan strain Hatchery	Emersion (complete drain)	3 min (for 9 months)	3.5 ng/ml ± 4.7 ($n = 10$)	26.4 ng/ml ± 3.2 (1 h; after 4.5 months)	Campbell et al., 1992
Males, maturation	Annan strain Hatchery	Confinement	2 weeks	1.8 ng/ml ± 0.2 ($n = 6$)	18.0 ng/ml ± 5.7 (after 2 weeks confinement)	Campbell et al., 1994
Males, maturation	n.a. Hatchery	Confinement	(a) 1 h	(a) < 8.0 ng/ml	(a) 71.7 ng/ml	Pottinger et al., 1995
			(b) 3 h	(b) < 2.5 ng/ml	(b) ~ 30 ng/ml	
			(c) 24 h	(c) ~ 65 ng/ml ($n = 48$)	(c) ~ 65 ng/ml (immediately after)	
Males, post-puberty	n.a. Hatchery	Confinement	(a) 1 h	(a) < 8.0 ng/ml	(a) 114.8 ng/ml	Pottinger et al., 1995
			(b) 3 h	(b) < 2.5 ng/ml	(b) ~ 50 ng/ml	
			(c) 24 h	(c) < 9.0 ng/ml ($n = 48$)	(c) ~ 100 ng/ml (immediately after)	
Males, maturation	Whitikau Stream Wild	(a) Barrier	(a) 0–14 h	6.1 ng/ml ± 0.8 ($n = 8$–11)	(a) 83.5 ng/ml ± 14	Clements, 1996
		(b) Cage	(b) 1 h		(b) 185.1 ng/ml ± 40	
		(c) Crowding	(c) 1 h		(c) 233.0 ng/ml ± 31	
Males, maturation	Stirling Strain (low response) Hatchery	Confinement	3 h		45.2 ng/ml ± 3.2 ($n = 75$)	Pottinger and Carrick, 1999
Males, maturation	Stirling Strain (high response) Hatchery	Confinement	3 h		69.0 ng/ml ± 5.2 ($n = 75$)	Pottinger and Carrick, 1999

[a] Quantified using a fluorimetric assay.

concentrations in rainbow trout eggs that were stressed up to 3 months prior to spawning. In fact, elevated levels of maternal cortisol at ovulation were not reflected in the concentrations in ovarian fluid or eggs (Contreras-Sanchez, 1995). Furthermore, the early developing embryo has the biochemical machinery to metabolize steroid hormones, including cortisol (Yeoh et al., 1996a,b), which suggests that regulation of maternally contributed factors occurs post-fertilization in the embryo. Implantation of cortisol into adult tilapia, *Oreochromis mossambicus*, resulted in reduced oocytes size and circulating testosterone and 17β-estradiol concentrations (Foo and Lam, 1993).

3.1.2. Immunosuppression and disease resistance

Unless killed outright by a stressful situation, fish tend to die due to secondary rather than primary effects of the stressor in situations where they cannot compensate adequately. One such secondary effect occurs when stressed fish succumb to pathogens that they could otherwise resist. Stress is known to be immunosuppressive in fish (see review by Schreck, 1996). For adult fish, an extreme example of this is to be found with Pacific salmon, *Oncorhynchus* spp., that all die after spawning. Maturation and spawning in Pacific salmon is accompanied by loss of pituitary control of the interrenal secretion of cortisol; concomitant with this is a decrease in the ability to clear cortisol from the circulation (Robertson and Wexler, 1957; Hane and Robertson, 1959; Robertson et al., 1961; Hane et al., 1966; Idler et al., 1963, 1966; Donaldson and Fagerlund, 1968, 1972; Dickhoff, 1989; Stein-Behrens and Saplosky, 1992). In senescent mammals cortisol down-regulates its own receptor centrally, thereby rendering negative feedback less sensitive Stein-Behrens and Saplosky (1992). The net effect is that plasma levels of cortisol become elevated and the fish succumb to Cushing's syndrome. Consequently, the fish either become infected with new pathogens or can no longer resist latent pathogens. Sustained elevated levels of cortisol seem to be the cause of the immunosuppression that renders the fish vulnerable to the pathogens. Any species of broodfish exposed to any stressor that elevates circulating concentrations of cortisol for more than a brief period of time could exhibit similar symptoms.

Cortisol (and stress) depresses the ability of fish leukocytes to form antibodies (Maule et al., 1987, 1989). This suppression seems to operate through a cortisol receptor-mediated system in the lymphocyte (Maule and Schreck, 1990a, 1991). Stress and cortisol also affect the numbers of leukocytes in immune organs (Maule and Schreck, 1990b). Adult fish of either sex may produce significant amounts of androgens such as testosterone during maturation that are immunosuppressive (Slater, 1991; Slater et al., 1995a) and appear to operate through a receptor-mediated system (Slater et al., 1995b). While cortisol may interfere with production of an essential cytokine (Tripp et al., 1987), androgens may be immunosuppressive by causing premature leukocyte death (Slater and Schreck, 1997). It should be noted that these conclusions about stress and the endocrine regulation of the immune system are based on studies employing juvenile tissue; work of this nature with adults is extremely limited.

Nevertheless, some research has linked cortisol to immune function in adults. In adult female chinook salmon, *O. tshawytscha*, numbers of antibody producing cells in peripheral blood were negatively correlated with cortisol and positively correlated to androgens and oestrogen; whereas no correlation was observed with progestin (Maule et

al., 1996). In that study, fish migrating upstream and those held under hatchery conditions at warmer, variable temperatures had elevated cortisol levels and a reduced capacity to elaborate antibody-producing leukocytes. When those fish were moved to cooler, constant temperature aquaculture conditions about a month before ovulation, cortisol levels decreased and antibody producing cell numbers increased and remained high until spawning. Glucocorticoid receptors in the leukocytes did not appear to vary over the period of maturation. Lysozyme activity, which seems responsive to stressors, is similar between primary and secondary circulations in early vitellogenesis but becomes lower in the blood in chinook salmon nearer to ovulation that had returned to a hatchery (Maule et al., 1996). Extending the conclusions from the above studies, late maturation-stage broodfish may be particularly vulnerable to stress-induced disease since resting testosterone and cortisol titres would be elevated, which could affect both specific and non-specific arms of the immune system.

3.2. Temporal scaling, gamete quality, and reproductive fitness

To be able to understand the effects of stressors on a particular broodstock, one must consider the time during gonadal development at which stress occurs and the severity and duration of the stressor. These factors may lead to completely different responses depending on the species' reproductive strategies and costs. Under stressful conditions, trade-offs between reproductive efforts, somatic growth and survival may occur. These trade-offs imply that under adverse conditions, a female can select between energy allocated for maintenance and somatic growth, or energy for reproduction. The most common trade-offs involve fecundity and life span. The considerable loss of body size or growth consequent to stressful conditions has been well documented for many species. Roff (1982) proposed two patterns of energy allocation during reproductive trade-offs: a) maintenance of body weight and adjustment in gamete production, and b) maintenance of constant numbers of eggs at the expense of somatic tissue. The former trade-off may result when stressors compromise the development of the ovary during vitellogenesis, which results in egg atresia and subsequent reabsorption. The second could happen when somatic tissue is severely affected during pre-spawning migration but number and quality of eggs remain constant. Egg number appears to evolve around selection for egg size (Fleming and Gross, 1990).

It is well established that environmental variables can affect timing of reproductive functions. The transition from juvenile to adult stage, puberty, can be difficult to describe; puberty in male fish was recently reviewed by Schulz and Goos, 1999). There is considerable plasticity for age and size of fish at sexual maturity in response to stress (Stearns and Crandall, 1984). *Ultimate* reproductive timing factors, particularly nutrition, can determine age to first maturity and also age at which and frequency of subsequent reproductive events in fish (Sadleir, 1973). For example, dietary protein was shown to be important for puberty and gamete quality in Nile tilapia, *Ore. niloticus* (Gunasekera et al., 1995). Nutritional stress experienced earlier in the life cycle can affect the timing of puberty, maturation as well as fecundity. With older-lived species, age at first spawning can be delayed by years by nutritional stress; similarly, the duration between spawning events can be prolonged by years due to nutritional deficits.

The mechanism by which nutritional state is translated into the physiology that establishes the organism's reproductive fate remains ill defined. How does the fish establish that it is "safe" to proceed with further reproductive development?

Environmental stressors and particularly nutrition can also affect realized fecundity and gamete quality. In general, larger females produce larger eggs, which give rise to progeny that are believed to have an ecological advantage over those from smaller eggs. Therefore, a stressor that affects growth may lead to the production of progeny that are already at a disadvantage because of their smaller size. The number of ripe eggs a female produces is also based on environmental quality. A female produces oogonia during early developmental stages and then during the process of oogenesis establishes the actual number of these that will start to mature and that will be ovulated, with the remainder being reabsorbed through atresia. Fish must balance the production of eggs against the requirements to maintain at least some minimal level of quality (size and content). How fish weigh the trade-off between these variables is unknown. Literature in this area includes discussions on reproductive tactics relative to egg size, fecundity and age at maturity (Hislop, 1984); resource allocation for somatic growth and gonad nutrient content (Iles, 1984; Encina and Granado-Lorencio, 1997); age-structuring, energy acquisition and fitness (Ware, 1984); the relation of hatching success to female condition (Laine and Rajasilta, 1999); and the importance of nutrients, particularly thiamine, in early embryo mortality syndrome (Hornung et al., 1998).

The most fundamental decision by a fish in the face of stress is whether or not to reproduce. If reproduction is to occur, then the fish must balance fecundity with egg quality. To clarify tactics adopted by fish with very different reproductive life histories, we compared Nile tilapia (multiple spawning—throughout the year) with rainbow trout (simple spawning—once per year at most (Table 2). The period of maturation when fish are exposed to a stressor appears important. Tilapia encountering stressors (disturbance and agitation) during early ovarian development delayed ovulation while those stressed during late vitellogenesis spawned immediately (Contreras-Sanchez, unpublished data). Similarly, ovulation occurred earlier when rainbow trout were exposed to a mild stressor during late vitellogenesis (Contreras-Sanchez et al., 1998). Interestingly, ovulation was also advanced if fish were mildly stressed during the whole vitellogenic period. The severity of the stressor also appears important. Trout under more severe stress (emersions) for 9 months prior to spawning produced small eggs and delayed ovulation (Campbell et al., 1992). Under a milder stress regime (disturbance once per day) during early vitellogenesis, rainbow trout also produced smaller eggs that varied in size, while there was no effect on mean egg size in fish stressed during late vitellogenesis (Contreras-Sanchez et al., 1998). The fish mildly stressed during the whole vitellogenic period showed no effect on mean egg size but the egg sizes were more heterogeneous. Only slightly more than one-half of the oocytes of female striped trumpeter, *Latris lineata*, that experienced frequent handling developed past the cortical alveoli/early vitellogenic stage; the mean volume of eggs produced each day was greater in stressed than non-stressed fish (Morehead et al., 2000).

Other teleosts have also been shown to employ quite varied reproductive strategies to cope with stress. For example, stress or the lack thereof seems to be involved in regulating sex reversal in hermaphroditic species where density, growth and social status

Table 2
The effects of stress at various times on reproductive traits of two species with differing reproductive strategies

	Tilapia, moderate stress (Contreras-Sanchez, unpublished data)	Rainbow trout, mild stress (Contreras-Sanchez et al., 1998)	Rainbow trout, severe stress
Stress during early vitellogenesis	Lower growth rate No spawning	Slight effect on growth rate No effect on spawning No effect on absolute fecundity Smaller eggs Large variation on egg size No effect on progeny survival	
Stress during late vitellogenesis	Fast spawning	No effect on growth rate Early spawning (2 weeks earlier) No effect on absolute fecundity No effect on egg size Large variation on egg weight No effect on progeny survival	No effect on size (Campbell et al., 1994) No effect on absolute fecundity Smaller eggs Lower progeny survival
Stress during entire vitellogenesic period	n.a.	Slight effect on growth rate Early spawning (2 weeks earlier) No effect on absolute fecundity No effect on egg size Large variation on egg weight No effect on progeny survival	No effect on size (Campbell et al., 1992) Delayed spawning (~ 3 weeks) No effect on absolute fecundity Smaller eggs Lower progeny survival

are important regulators of phenotypic sex (Robertson, 1972; Shapiro, 1981; Ross, 1987; Hourigan, 1986). For live-bearers, there seems to be only anecdotal information indicating that parturition is accelerated by stress. Fish of two genera of poeciliid, *Poecilia* and *Phallichtys*, were observed on numerous occasions to release their young while unstressed individuals at similar stages of pregnancy did not (Contreras-Sanchez, unpublished data). Shifts in reproductive tactics under stressful situations are probably important for optimizing reproductive fitness for fish in the wild, and understanding of such processes is obviously important for management of wild and hatchery stocks.

3.3. Stress and progeny quality

Unfortunately, other than nutritive effects mentioned earlier, only limited information exists on how stress experienced by broodfish affects their progeny. Campbell et al. (1992, 1994) found that relatively severe stress in rainbow trout for prolonged times can affect progeny survival. This reduced progeny viability could be due to limited energetic reserves allocated to the eggs as well as mechanical damage caused by the specific stressor. In contrast, progeny from females that experienced mild stress during various stages of vitellogenesis, by contrast, did not suffer abnormal mortality (Contreras-Sanchez et al., 1998). However, one can surmise that smaller eggs and hence smaller hatchlings resulting from some stressful situations, as found by Contreras-Sanchez et al. (1998), could be maladaptive in the wild. An interesting study by McCormick (1998) suggested that stress induced by the presence of predators and to a lesser extent conspecifics in

female ambon damselfish resulted in eggs with higher cortisol concentrations and that there was an inverse correlation between levels of this hormone in eggs and egg size. The implications of this work are that behavioural interactions and stress incurred by broodfish can affect progeny quality.

4. A progeny-protecting system: a modest proposal

We suggest that the females have a mechanism that allows them to protect or buffer their eggs from the deleterious effects of stress. At a gross level, such buffering can be exemplified in the trade-off between fecundity and egg quality when a female reabsorbs some eggs to spare energy or nutrients for the remainder during nutritionally stressing times. However, there are likely numerous maternal mechanisms that also spare individual eggs from potentially maladaptive physiological responses induced by stressors. Based on the extant evidence, two main avenues where the stressed maternal system can maintain the quality of eggs are proposed: (1) the quantity and nature of substances transferred from the female to the egg, and (2) the timing of maturation and ovulation (Fig. 1).

Maternal transfer of vitellogenin, lipid and other nutrients are essential during egg maturation. The composition and accumulation of yolk in teleost eggs were discussed in detail in the excellent review by Wiegand (1996). However, while certain levels of compounds such as hormones could be important for regulating development (Lam, 1985; Brown et al., 1989; Brown and Bern, 1989; Feist et al., 1990; Schreck et al., 1991; Ayson and Lam, 1993; Yeoh et al., 1996a,b), an overabundance could be detrimental. For example, cortisol is secreted when fish are stressed and it is anti-developmental, anti-growth (Schreck, 1992), and immunosuppressive (Schreck, 1996). Furthermore, Cloud (1981) found that hatching was accelerated when medaka, *Oryzias latipes*, eggs were incubated with deoxycorticosterone. It is thus not surprising that at ovulation both stressed as well as unstressed rainbow trout had 17 times less cortisol in ovarian fluid and 30 times less in eggs than in their circulation (Contreras-Sanchez, 1995). It is thus likely that the female protects the eggs from hypercortisolism.

We postulate that this could be accomplished by three likely mechanisms. First, the cortisol stress response of ripe females appears to be attenuated. As mentioned earlier, we found that the physiological stress response in rainbow trout during early and late vitellogenesis to mildly stressful situations is fast and brief. Cortisol levels reached its highest value 20 min after application of the stress, and returned to resting values 40 min after the stress. Thus, although the lipophilic nature of eggs would allow them to readily take up cortisol, this is limited by the brevity of the circulatory increase in cortisol. Second, the presence of binding proteins may keep the majority of steroid in the maternal circulation. During ovarian maturation in rainbow trout, only 22% of the cortisol was found to be “free”, as increasing concentrations of corticosteroid binding protein bound approximately 45% of the hormone, with another 33% bound to albumins (Caldwell et al., 1991). Third, cortisol metabolizing enzymes in the follicle could convert cortisol leaving the circulation into its inactive metabolite, cortisone, at the follicle. It is not known whether the theca or granulosa possesses the appropriate

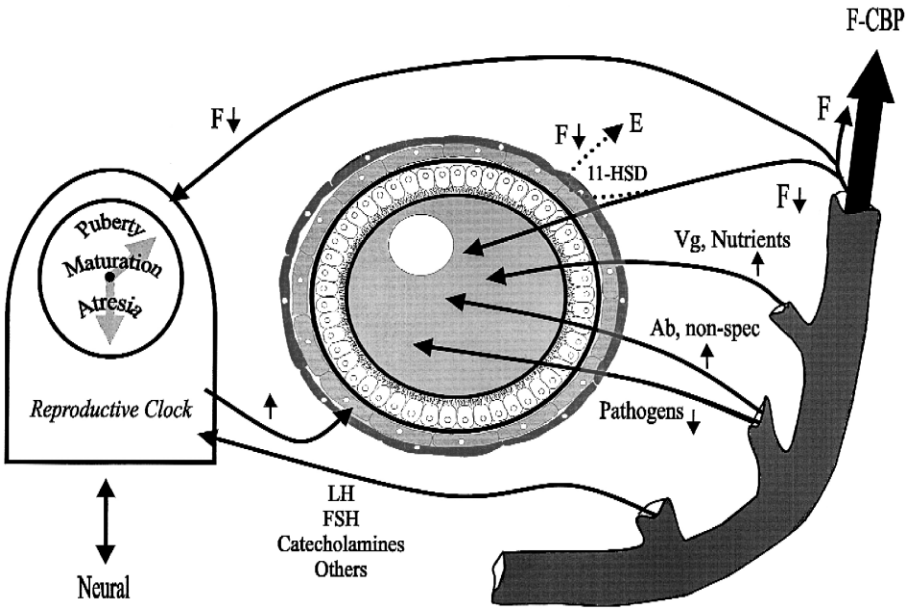

Fig. 1. Conceptualization of a buffering system that can maintain egg quality during resting and stressed states. Short up and down arrows indicate the effects of buffering on maintaining levels of some factor or process above some minimum or below some maximum (homeostasis or allostasis). The vascular system provides numerous factors to the developing egg and also to the physiological systems controlling timing of reproductive events (Reproductive Clock). For example, an overload of cortisol (F) in the egg is prevented by corticosteroid binding protein (CBP), brevity of the stress response in mature fish affecting the clock, or via enzymatic buffering converting F to cortisone (E). Nutrients, (e.g., vitellogenin, Vg) and specific antibodies (Ab) and non-specific immune factors help protect against pathogens. The clock can spare energy and nutrients for eggs via timing of ovarian atresia or by optimizing spawning timing. Central (neural) control of the Reproductive Clock is via direct or indirect (e.g., endocrine) mechanisms.

11-hydroxysteroid dehydrogenase (HSD) system found in other fish tissues (e.g., see Colombo et al., 1972; Donaldson and Fagerlund, 1972; Kime, 1978a,b; Truscott, 1979; Patiño et al., 1987; Pottinger et al., 1992). Nor is it known if cortisol is produced by follicular cells such that it could enter the egg via a paracrine pathway; follicular tissue of *Gillichthys mirabilis*, *Leptocottus armatus*, and *Microgadus proximus* are capable of producing 11-deoxygenated corticosteroids [e.g., 11-deoxycorticosterone and 11-deoxycortisol (Columbo et al., 1973); see also van Tienhoven (1983) for a review of steroid production in gonads of non-mammalian vertebrates]. The binding of the receptor for the maturation-inducing steroid 17α,20β,21-trihydroxy-4-pregnen-3-one in ovarian tissue of the spotted sea trout, *Cynoscion nebulosus*, with 11-deoxycorticosterone (Patiño and Thomas 1990), presupposes a biological effect. Mature rainbow trout apparently also have plasma-binding proteins that have relatively high affinity for the 11-deoxygenated corticosteroids (Fostier and Breton, 1975). It is also unknown if cortisol or other corticosteroids could leave an egg and return into the circulation. However, it is possible that cortisol is prevented from entering the egg by enzymatic

buffering involving the production of corticosterone. Monder and White (1993) provided a thorough state of the art regarding 11β-HSD, including fish. They point out that this enzyme system likely protects the Leydig cell of the testis from inhibitory effects of glucocorticoids.

It is known that stress predisposes fish to infection by pathogenic agents (Schreck, 1996). However, it remains unclear if exposure of broodstock to stressors affects the likelihood that progeny will become infected with pathogens. Maternal protection of eggs from infection must exist since vertical transmission of microparasites is not the rule, although some bacteria such as *Renibacterium salmoninarum*, which cause bacterial kidney disease (Evelyn et al., 1984; Lee and Evelyn, 1989; Elliott et al., 1989; Brown et al., 1990), and viruses, like infectious pancreatic necrosis virus (Bootland et al., 1991), can be passed from the female to eggs (see also review by Brock and Bullis, in press, this volume). Mechanisms by which infection of eggs is limited or prevented are not known to exist, although one could speculate that the impermeability of the egg itself confers some protection. It is known that the female confers some form of protection or immunity to the embryo by loading unfertilized eggs with maternally derived immune protection and other non-specific defense mechanisms such as lectins and hemagglutinins (Brown et al., 1990, 1994; Yousif et al., 1994a,b, 1995; Yano, 1996; Tatner, 1996), which could buffer developing embryos from pathogenic insults consequent to stress of female broodfish.

Buffering might also take place with regard to timing of key reproductive events during development (the 'Reproductive Clock'). The timing of puberty, maturation and atretic events are influenced by stressors as discussed in Section 3.2. Direct connections between physiological stress responses and factors moderating the timing of reproductive events are unclear. There could be an obvious connection between the stress-induced production of catecholamines (Mazeaud et al., 1977) and the role of these hormones in ovulation (Jalabert, 1976). Research has also suggested that corticosteroids in vivo and in vitro could induce maturation and ovulation in the catfish, *Heteropneustes fossilis* (Goswami and Sundararaj, 1971a,b, 1974) and that corticosteroidogenesis in follicular tissue of medaka is required for gonadotropin-induced ovulation (Hirose, 1976).

Much of the timing of reproductive events is controlled by the gonadotropins LH and FSH. While it is not known how stress might affect these hormones, acute stressors have been found to increase circulating levels in brown trout, *Salmo trutta* (Pickering et al., 1987; Sumpter et al., 1987) or decrease concentrations in white suckers, *Catostomus commersoni* (Stacey et al., 1984). More prolonged stress might cause gonadotropin levels to decrease below normal as evidenced in rainbow trout that did not resume feeding after aortic catheterization (Zohar, 1980). A crude gonadotropin preparation was found to cause interrenal secretion of cortisol in vitro in coho salmon (Schreck et al., 1989), indicating a potential direct link between gonadotropins and stress hormones. Stressors clearly depress the sex hormones such as testosterone, 11-ketotestosterone, and 17β-estradiol in both male and female teleosts such as *C. nebulosus* (Safford and Thomas, 1987), rainbow and brown trout (Pickering et al., 1987; Sumpter et al., 1987; Pankhurst and Dedual, 1994), snapper, *Pagrus auratus* (Carragher and Pankhurst, 1991), red gurnard, *Chelidonichthys kumu* (Clearwater, 1992), and white sucker (Van

Der Kraak et al., 1992; McMaster et al., 1994; Jardine et al., 1996). The maturational steroid 17β, 20β-dihydroxyprogesterone was elevated in stressed female snapper (Carragher and Pankhurst, 1991). The nature and chronology of the stressor seems to determine if concentrations of these steroids are affected, for some negative data have also been reported (Van Der Kraak et al., 1992; McMaster et al., 1994; Pankhurst and Dedual, 1994). Sumpter (1997) reviewed the effects of stress on some other hormones.

We could also postulate that neural and other mechanisms might affect the reproductive clock. These and hormonal timing factors could influence early stages of egg maturation by affecting follicular processes or cause ovulation and spawning. During early oogenesis or maturation, the reproductive clock could buffer some eggs from energetic or nutritional deficits by causing atresia of others. Near full maturity, reproductive success could be optimized by regulating timing of spawning because, depending on life history of the species and the nature of the stressor, ecological advantage could be gained by either advancing or delaying spawning.

5. Concluding remarks

In examining the impact of stressors on broodstock, it is helpful to list those attributes of broodstock that are desirable. Bromage (1995) examined these attributes from an aquaculture perspective. For both fish in the wild and for those under culture conditions, broodstock should (1) have optimal growth to maturity, (2) be capable of undergoing maturation (physiologically and/or behaviorally) at the optimal time (developmentally and seasonally), (3) have a high likelihood of surviving through spawning, and (4) produce gametes in optimal numbers, of optimal sizes (in the case of eggs), and of optimal quality. The genetic make-up of the fish provides the blueprint on which these performance characteristics are based; however, specific conditions (e.g., stress, nutritional status) act to modify these performance characteristics further. These modifications can either be adaptive, maladaptive, or neutral.

Knowledge of how a stressor might affect the physiology of a species can help in development of management tactics that lessen the impact of a stressor or even in the development of therapeutants. Knowing that stressors can have varying effects depending on when in the reproductive process they are experienced can be relevant in optimizing management or culture objectives. For example, issues such as reduction in between-individual variation for aquaculture or maintenance of extant variation in reproductive timing for bioconservation can be important. Understanding the physiological response of fish to stressors can be valuable for maintaining reproductive fitness of fish. Pathologic insult to early developmental stages could be moderated by disease-preventive or therapeutic agents identified with the knowledge of maternal buffering systems and the effects of stress.

References

Ayson, F.G., Lam, T.J., 1993. Thyroxin injection of female rabbitfish (*Siganus guttatus*) broodstock: changes in thyroid hormone levels in plasma, eggs, and yolk-sac larvae, and its effect on larval growth and survival. Aquaculture 109, 83–93.

Balon, E.K., 1975. Reproductive guilds of fishes: a proposal and definition. J. Fish. Res. Board Can. 32, 821–864.

Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1, 3–26.

Bootland, L.M., Dobos, P., Stevenson, R.M.W., 1991. The IPNV carrier state and demonstration of vertical transmission in experimentally infected brook trout. Dis. Aquat. Org. 10, 13–21.

Brock, J.A., Bullis, R., in press. Disease prevention and control for gametes and embryos of fish and marine shrimp. Aquaculture.

Bromage, N., 1995. Broodstock management and seed quality–general considerations. In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford, England, pp. 1–24.

Brown, C.L., Bern, H.A., 1989. Hormones in early development, with special reference to teleost fish. In: Scanes, C.G., Scheibman, M.P. (Eds.), Hormones in Development, Maturation and Senescence of Neuroendocrine Systems. Academic Press, San Diego, pp. 189–306.

Brown, C.L., Doroshov, S.I., Cochran, M.D., Bern, H.A., 1989. Enhanced survival in striped bass fingerlings after maternal triiodothyronine treatment. Fish. Physiol. Biochem. 7, 295–299.

Brown, L.L., Albright, L.J., Evelyn, T.P.T., 1990. Control of vertical transmission of *Renebacterium salmoninarum* by injection of antibodies into maturing female coho salmon *Oncorhynchus kisutch*. Dis. Aquat. Org. 9, 127–131.

Brown, L.L., Evelyn, T.P.T., Iwama, G.K., 1994. Vertical transfer of passive immunity from coho salmon via their eggs. Int. Symp. Aquatic Animal Health, Seattle, WA. University of California, Davis, p. W-6.1.

Bry, C., 1985. Plasma cortisol levels of female rainbow trout (*Salmo gairdneri*) at the end of the reproductive cycle: Relationship with oocyte stages. Gen. Comp. Endocrinol. 57, 47–52.

Bry, C., Zohar, Y., 1980. Dorsal aorta catheterization in rainbow trout (*Salmo gairdneri*): II. Glucocorticoid levels, hematological data and resumption of feeding for five days after surgery. Reprod. Nutr. Dev. 20, 1825–1834.

Caldwell, C.A., Kattrsh, H.G., Strange, R.J., 1991. Distribution of cortisol among its free and protein-bound fractions in rainbow trout (*Oncorhynchus mykiss*): evidence of control by sexual maturation. Comp. Biochem. Physiol. 99A, 593–595.

Campbell, P.M., Pottinger, T.G., Sumpter, J.P., 1992. Stress reduces the quality of gametes produced by rainbow trout. Biol. Reprod. 47, 1140–1150.

Campbell, P.M., Pottinger, T.G., Sumpter, J.P., 1994. Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. Aquaculture 120, 151–169.

Carragher, J.F., Sumpter, J.P., 1990. The effect of cortisol on the secretion of sex steroids from cultured ovarian follicles of rainbow trout. Gen. Comp. Endocrinol. 77, 403–407.

Carragher, J.F., Pankhurst, N.W., 1991. Stress and reproduction in a commercially important marine fish, *Pagrus auratus* (Sparidae). In: Scott, A.P., Sumpter, J.P., Kime, D.E., Rolfe, M.S. (Eds.), Reproductive Physiology of Fish 1991. FishSymp 91, Sheffield, pp. 253–255.

Clearwater, S.J., 1992. Reproductive biology and response to capture stress of the red gurnard *Chelidonichthys kumu* (Family: Triglidae). Master's Thesis, Univ. Auckland, 96 pp.

Clements, S.P., 1996. Primary and secondary stress responses to trapping in wild rainbow trout (*Oncorhynchus mykiss*). MS Thesis, University of Waikato, 112 pp.

Cloud, J.G., 1981. Deoxycorticosterone-induced precocious hatching of teleost embryos. J. Exp. Zool. 216, 197–199.

Colombo, L., Pesavento, S., Johnson, D.W., 1972. Patterns of steroid metabolism in teleost and ganoid fishes. Gen. Comp. Endocrinol., Suppl. 3, 245–253.

Columbo, L., Bern, H.A., Pieprzyk, J., Johnson, D.W., 1973. Biosynthesis of 11-deoxycorticosteoids by teleost ovaries and discussion of their possible role in oocytes maturation and ovulation. Gen. Comp. Endocrinol. 21, 168–178.

Contreras-Sanchez, W.M., 1995. Effects of stress on the reproductive performance and physiology of rainbow trout (*Oncorhynchus mykiss*). MS thesis, Oregon State University, 60 pp.

Contreras-Sanchez, W.M., Schreck, C.B., Fitzpatrick, M.S., Pereira, C.B., 1998. Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). Biol. Reprod. 58, 439–447.

Dauprat, P., Dalle, M., Delost, P., 1990. Effects of neurotrophic stress on maternal metabolism and binding of plasma cortisol in late pregnant guinea pigs and their fetuses. J. Dev. Physiol. 13, 13–16.

Dickhoff, W.W., 1989. Salmonids and annual fishes: death after sex. In: Schreibman, M.P., Scanes, C.G. (Eds.), Development, Maturation, and Scenescence of Neuroendocrine Systems: A Comparative Approach. Academic Press, New York, pp. 253–266.

Donaldson, E.M., 1981. The pituitary–interrenal axis as an indicator of stress in fish. In: Pickering, A.D. (Ed.), Stress in Fish. Academic Press, London, pp. 11–47.

Donaldson, E.M., Fagerlund, U.H.M., 1968. Changes in the cortisol dynamics of sockeye salmon (*Oncorhyhus nerka*) resulting from sexual maturation. Gen. Comp. Endocrinol. 11, 552–561.

Donaldson, E.M., Fagerlund, U.H.M., 1972. Corticosteroid dynamics in Pacific salmon. Gen. Comp. Endocrinol. 3, 254–265.

Elliott, D.G., Pascho, R.J., Bullock, G.L., 1989. Developments in the control of bacterial kidney disease of salmonid fishes. Dis. Aquat. Org. 6, 201–215.

Encina, L., Granado-Lorencio, C., 1997. Seasonal variations in the physiological status of energy content and somatic and reproductive tissues of chub. J. Fish Biol. 50, 511–522.

Evelyn, T.P.T., Ketcheson, J.E., Prosperi-porta, L., 1984. Further evidence for the presence of *Renibacterium salmoninarum* in salmonid eggs and for the failure of povidone–iodine to reduce the intra-ovum infection rate in water-hardened eggs. J. Fish Dis. 7, 173–182.

Feist, G., Schreck, C.B., Fitzpatrick, M.S., Redding, J.M., 1990. Sex steroid profiles of coho salmon (*Oncorhynchus kisutch*) during early development and sexual differentiation. Gen. Comp. Endocrinol. 80, 299–313.

Fevolden, S.E., Refstie, T., Gjerde, B., 1993. Genetic and phenotypic parameters for cortisol and glucose stress response in Atlantic salmon and rainbow trout. Aquaculture 118, 205–216.

Fleming, I.A., Gross, M.R., 1990. Latitudinal clines: a trade-off between egg number and size in pacific salmon. Ecology 71, 1–11.

Foo, J.T.W., Lam, T.J., 1993. Retardation of ovarian growth and depression of serum steroid levels in the tilapia, *Oreochromis mossambicus*, by cortisol implantation. Aquaculture 115, 133–143.

Fostier, A., Breton, B., 1975. Binding of steroids by plasma of a teleosts: the rainbow trout, *Salmo gairdnerii*. J. Steroid Biochem. 6, 345–351.

Goswami, S.V., Sundararaj, B.I., 1971a. Temporal effects of ovine luteinizing hormone and desoxycorticosterone acetate on maturation and ovulation of oocytes of the catfish, *Heteropneustes fossilis* (Bloch): an in vivo and in vitro study. J. Exp. Zool. 178, 457–466.

Goswami, S.V., Sundararaj, B.I., 1971b. In vitro maturation and ovulation of oocytes of the catfish, *Heteropneustes fossilis* (Bloch): effects of mammalian hypophyseal hormones, catfish pituitary homogenate, steroid precursors and metabolites, and gonadal and adrenocotical steroids. J. Exp. Zool. 178, 467–478.

Goswami, S.V., Sundararaj, B.I., 1974. Effects of C_{18}, C_{19}, and C_{21} steroids on in vitro maturation of oocytes of the catfish, *Heteropneustes fossilis* (Bloch). Gen. Comp. Endocrinol. 23, 282–285.

Greenberg, N., Wingfield, J.C., 1987. Stress and reproduction: reciprocal relationships. In: Norris, D.O., Jones, R.E. (Eds.), Hormones and Reproduction in Fishes, Amphibians and Reptiles. Plenum, New York, pp. 451–505.

Gunasekera, R.M., Shim, K.F., Lam, T.J., 1995. Effect of dietary protein level on puberty, oocytes growth and egg chemical composition in the tilapia *Oreochromis niloticus* (L.). Aquaculture 134, 169–183.

Hane, S., Robertson, O.H., 1959. Changes in plasma 17-hydroxycorticosteroids accompaning sexual maturation and spawning of the Pacific salmon (*Oncorhynchus tschawytscha*) and rainbow trout (*Salmo gairdneri*). Pac. Natl. Acad. Sci. US 45, 886–893.

Hane, S., Robertson, O.H., Wexler, B.C., Krupp, A., 1966. Adrenocortical response to stress and ACTH in Pacific salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*) at successive stages in the sexual cycle. Endocrinology 78, 791–800.

Hill, C.F., Fromm, P.O., 1968. Response of the interrenal gland of rainbow trout (*Salmo gairdneri*) to stress. Gen. Comp. Endocrinol. 11, 69–77.

Hirose, K., 1976. Endocrine control of ovulation in medaka (*Oryzias latipes*) and ayu (*Plecoglossus altivelis*). J. Fish. Res. Board Can. 33, 989–994.

Hislop, J.R.G., 1984. A comparison of the reproductive tactics and strategies of cod, haddock, whiting and

Norway pout in the North Sea. In: Potts, G.W., Wootton, R.J. (Eds.), Fish Reproduction: Strategies and Tactics. Academic Press, London, pp. 311–329.
Hornung, M.W., Miller, L., Peterson, R.E., Marcquenski, S., Brown, S.B., 1998. Efficacy of thiamine, astaxanthin, α-oarotene, and thyroxine treatments in reducing early mortality syndrome in Lake Michigan salmonid embryos. Am. Fish. Soc. Symp. 21, 124–134.
Hourigan, T.F., 1986. A comparison of haremic social systems in two reef fishes. In: Crickamer, L.C. (Ed.), Behavioral Ecology and Population Biology. Privat, I.E.C., Toulouse, pp. 23–28.
Idler, D.R., Truscott, B., in collaboration with Freeman, H.C., Chang, G., Schmidt, P.J., Ronald, A.P., 1963. In vivo metabolism of steroid hormones by sockeye salmon: A. Impaired hormone clearance in mature and spawned Pacific salmon (*Oncorhynchus nerka*); B. Precursors of 11-ketotestosterone. Can. J. Biochem. Physiol. 41, 875–887.
Idler, D.R., Schmidt, P.J., Truscott, B., Freeman, H.C., 1966. Impaired hormone metabolism in relation to spawning and death of salmon. Food Sci. 2, 211–218.
Iles, T.D., 1984. Allocation of resources to gonad and soma in Atlantic herring *Clupea harengus* L. In: Potts, G.W., Wootton, R.J. (Eds.), Fish Reproduction: Strategies and Tactics. Academic Press, London, pp. 331–347.
Jalabert, B., 1976. In vitro oocyte maturation and ovulation in rainbow trout (*Salmo gairdneri*), northern pike (*Esox lucius*), and goldfish (*Carassius auratus*). J. Fish Res. Board Can. 33, 974–988.
Jardine, J.J., Van Der Kraak, G.J., Munkittrick, K.R., 1996. Capture and confinement stress in white sucker exposed to bleached kraft pulp mill effluent. Ecotoxicol. Environ. Saf. 33, 287–298.
Kime, D.E., 1978a. Catabolism of the adrenocortical hormones. In: Jones, C., Henderson, I., Henderson, I.W. (Eds.), General, Comparative and Clinical Endocrinology of the Adrenal Cortex. Academic Press, New York, pp. 265–290.
Kime, D.E., 1978b. The hepatic catabolism of cortisol in teleost fish: adrenal origin of 11-oxotestosterone precursors. Gen. Comp. Endocrinol. 35, 322–328.
Laine, P., Rajasilta, M., 1999. The hatching success of Baltic herring eggs and its relation to female condition. J. Exp. Mar. Biol. Ecol. 237, 61–73.
Lam, T.J., 1985. Role of thyroid hormone on larval development in fish. In: Lofts, B., Holms, W.N. (Eds.), Current Trends in Comparative Endocrinology. Hong Kong Univ. Press, Hong Kong, pp. 481–485.
Lee, E.G.H., Evelyn, T.P.T., 1989. Effect of *Renibacterium salmoninarum* levels in the ovarian fluid of spawning chinook salmon on the prevalence of the pathogen in their eggs and progeny. Dis. Aquat. Org. 7, 179–184.
Maule, A.G., Schreck, C.B., 1990a. Changes in numbers of leucocytes in immune organs of juvenile coho salmon (*Oncorhynchus kisutch*) after acute stress or cortisol treatment. J. Aquat. Anim. Health 2, 298–304.
Maule, A.G., Schreck, C.B., 1990b. The glucocorticoid receptors in leukocytes and gill of juvenile coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 77, 448–455.
Maule, A.G., Schreck, C.B., 1991. Stress and cortisol treatment changed affinity and number of glucocorticoid receptors in leukocytes and gill of coho salmon. Gen. Comp. Endocrinol. 84, 83–93.
Maule, A.G., Schreck, C.B., Kaattari, S.L., 1987. Changes in the immune system of coho salmon (*Oncorhynchus kisutch*) during the parr–smolt transformation and after implantation of cortisol. Can. J. Fish. Aquat. Sci. 44, 161–166.
Maule, A.G., Tripp, R.A., Kaattari, S.L., Schreck, C.B., 1989. Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). J. Endocrinol. 120, 135–142.
Maule, A.G., Schrock, R., Slater, C., Fitzpatrick, M.S., Schreck, C.B., 1996. Immune and endocrine responses of adult chinook salmon during freshwater immigration and sexual maturation. Fish Shell. Immunol. 6, 221–233.
Mazeaud, M.M., Mazeaud, F., Donaldson, E.M., 1977. Primary and secondary effects of stress in fish: Some new data with a general review. Trans. Am. Fish. Soc. 106, 201–212.
McCormick, M.I., 1998. Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. Ecology 79, 1873–1883.
McEwen, B.S., 1998. Protective and damaging effects of stress mediators. N. Engl. J. Med. 338, 171–179.
McMaster, M.E., Munkittrick, K.R., Luxon, P.L., Van Der Kraak, G.J., 1994. Impact of low level sampling stress on interpretation of physiological responses of white sucker exposed to effluent from a bleached kraft mill. Ecotoxicol. Environ. Saf. 27, 251–264.

Monder, C., White, P.C., 1993. 11β-Hydroxysteroid dehydrogenase. Vitam. Horm. 47, 187–271.
Morehead, D.T., Ritar, A.J., Pankhurst, N.W., 2000. Effect of consecutive 9- or 12-month photothermal cycles and handling on sex steroid levels, oocyte development, and reproductive performance in female striped trumpeter *Latris lineata* (Latrididae). Aquaculture 189, 293–305.
Pagnotta, A., Brooks, L., Milligan, L., 1994. The potential regulatory roles of cortisol in recovery from exhaustive exercise in rainbow trout. Can. J. Zool. 72, 2136–2142.
Pankhurst, N.W., 1998. Further evidence of the equivocal effects of cortisol on in vitro steroidogenesis by ovarian follicles of rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. 19, 315–323.
Pankhurst, N.W., Dedual, M., 1994. Effects of capture and recovery on plasma levels of cortisol, lactate and gonadal steroids in a natural population of rainbow trout. J. Fish Biol. 45, 1013–1025.
Pankhurst, N.W., Van Der Kraak, G., 1997. Effects of stress on reproduction and growth of fish. In: Iwama, G.K., Sumpter, J., Pickering, A.D., Schreck, C.B. (Eds.), Fish Stress and Health in Aquaculture. Cambridge Univ. Press, Cambridge, pp. 73–93.
Pankhurst, N.W., Van Der Kraak, G., Peter, R.E., 1995. Evidence that the inhibitory effects of stress on reproduction in teleost fish are not mediated by the action of cortisol on ovarian steroidogenesis. Gen. Comp. Endocrinol. 99, 249–257.
Patiño, R., Thomas, P., 1990. Characterization of membrane receptor activity for 17α,20β, 21-trihydroxy 4-pregnen-3-one in ovaries of spotted sea trout (*Cynoscion nebulosus*). Gen. Comp. Endocrinol. 78, 204–217.
Patiño, R., Redding, J.M., Schreck, C.B., 1987. Interrenal secretion of corticosteroids and plasma cortisol and cortisone concentrations after acute stress and during seawater acclimation in juvenile coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 68, 431–439.
Pickering, A.D., 1981. Introduction: the concept of biological stress. In: Pickering, A.D. (Ed.), Stress and Fish. Academic Press, London, pp. 1–9.
Pickering, A.D., Pottinger, T.G., Carragher, J., Sumpter, J.P., 1987. The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. Gen. Comp. Endocinol. 68, 249–259.
Pottinger, T.G., Carrick, T.R., 1999. Modification of the plasma cortisol response to stress in rainbow trout. Gen. Comp. Endocrinol. 16, 122–132.
Pottinger, T.G., Moran, T.A., 1993. Differences in plasma cortisol and cortisone dynamics during stress in two strains of rainbow trout (*Oncorhynchus mykiss*). J. Fish Biol. 43, 121–130.
Pottinger, T.G., Pickering, A.D., Hurley, M.A., 1992. Consistency of the stress response of individuals of two strains of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 103, 275–289.
Pottinger, T.G., Knudsen, F.R., Wilson, J., 1994. Stress induced changes in the affinity and abundance of cytosolic cortisol binding sites in the liver of rainbow trout, *Oncorhynchus mykiss* (Walbaun), are not accompanied by changes in measurable nuclear binding. Fish Physiol. Biochem. 12, 499–511.
Pottinger, T.G., Balm, P.H.M., Pickering, A.D., 1995. Sexual maturity modifies the responsiveness of the pituitary–interrenal axis to stress in male rainbow trout. Gen. Comp. Endocrinol. 98, 311–320.
Robertson, D.R., 1972. Social control of sex reversal in a coral-reef fish. Sci. 177, 1007–1009.
Robertson, O.H., Wexler, B.C., 1957. Pituitary degeneration and adrenal tissue hyperplasia in spawning Pacific salmon. Science 125, 1295–1296.
Robertson, O.H., Krupp, M.A., Thomas, S.F., Favour, C.B., Hane, S., Wexler, B.C., 1961. Hyperadrenocorticism in spawning, migratory, and non-migratory rainbow trout (*Salmo gairdnerii*); comparison with Pacific salmon (Genus *Oncorhynchus*). Gen. Comp. Endocrinol. 1, 473–484.
Roff, D.A., 1982. Reproductive strategies in flatfish: a first synthesis. Can. J. Fish. Aquat. Sci. 39, 1686–1698.
Ross, R.M., 1987. Sex-change linked growth acceleration in a coral-reef fish, *Thalassoma duperrey*. J. Exp. Zool. 244, 455–461.
Sadleir, R.M.F.S., 1973. The Reproduction of Vertebrates. Academic Press, New York.
Safford, S.E., Thomas, P., 1987. Effects of capture and handling on circulating levels of gonadal steroids and cortisol in spotted seatrout, *Cynoscion nebulosus*. In: Idler, D.R., Crim, L.W., Walsh, J.M. (Eds.), Proceedings of the Third International Symposium on Reproductive Physilogy of Fish, St. John's.
Schreck, C.B., 1981. Stress and compensation in teleostean fishes: response to social and physical factors. In: Pickering, A.D. (Ed.), Stress and Fish. Academic Press, London, pp. 295–321.
Schreck, C.B., 1992. Glucocorticoids: metabolism, growth, and development. In: Schreibman, M.P., Scanes,

C.G., Pang, P.K.T. (Eds.), The Endocrinology of Growth, Development and Metabolism in Vertebrates. Academic Press, New York, pp. 367–392.
Schreck, C.B., 1996. Immunomodulation: endogenous factors. In: Iwama, G., Nakanishi, T. (Eds.), The Fish Immune System: Organism, Pathogen, and Environment. Academic Press, London, pp. 311–337.
Schreck, C.B., 2000. Accumulation and long-term effects of stress. In: Moberg, G.P., Mench, J.A. (Eds.), The Biology of Animal Stress: Assessment and Implications for Welfare. CAB International, Wallingford.
Schreck, C.B., Bradford, C.S., Fitzpatrick, M.S., Patiño, R., 1989. Regulation of the interrenal in fishes: non-classical control mechanisms. Fish Physiol. Biochem. 7, 259–265.
Schreck, C.B., Fitzpatrick, M.S., Feist, G.W., Yeoh, C.-G., 1991. Steroids: developmental continuum between mother and offspring. In: Scott, A.P., Sumpter, J.P., Kime, D.E., Rolfe, M.S. (Eds.), Proceedings of the 4th International Symposium on the Reproductive Physiology of Fish, FishSymp 91, Sheffield, pp. 256–258.
Schreck, C.B., Olla, B.L., Davis, M.W., 1997. Behavioral responses to stress. In: Iwama, G.W., Sumpter, J., Pickering, A.D., Schreck, C.B. (Eds.), Fish Stress and Health in Aquaculture. Cambridge Univ. Press, Cambridge, pp. 745–770.
Schulz, R.W., Goos, H.J.Th., 1999. Puberty in male fish: concepts and recent developments with special reference to the African catfish (*Clarias gariepinus*). Aquaculture 177, 5–12.
Selye, H., 1950. Stress and the general adaptation syndrome. Br. Med. J. 1, 1383–1392.
Selye, H., 1973. The evolution of stress concept. Am. Sci. 61, 692–699.
Shapiro, D.Y., 1981. Intragroup behavioural changes and the initiation of sex reversal in a coral reef fish in the laboratory. Anim. Behav. 29, 1199–1212.
Slater, C., 1991. Sex steroids, gonadotropins and effects on the immune response in maturing spring chinook salmon (*Oncorhynchus tshawytscha*). MS Thesis, Oregon State University, 71 pp.
Slater, C.H., Schreck, C.B., 1997. Physiological levels of testosterone kill salmonid leukocytes, in vitro. Gen. Comp. Endocrinol. 106, 113–119.
Slater, C.H., Fitzpatrick, M.S., Schreck, C.B., 1995a. Androgens and immunocompetence in salmonids: specific binding in and reduced immunocompetence of salmonid lymphocytes exposed to natural and synthetic androgens. Aquaculture 136, 363–370.
Slater, C.H., Fitzpatrick, M.S., Schreck, C.B., 1995b. Characterization of an androgen receptor in salmonid lymphocytes: possible link to androgen induced immunosuppression. Gen. Comp. Endocrinol. 100, 218–225.
Stacey, N.E., McKenzi, D.S., Marchant, T.A., Kyle, A.L., Peter, R.E., 1984. Endocrine changes during natural spawning in the white sucker *Catostomus commersoni*: I. Gonadotropin, growth hormone, and thyroid hormones. Gen. Comp. Endocrinol. 56, 333–348.
Stearns, S.C., Crandall, R.E., 1984. Plasticity for age and size at sexual maturity: a life history response to unavoidable stress. In: Potts, G.W., Wootton, R.J. (Eds.), Fish Reproduction: Strategies and Tactics. Academic Press, London, pp. 13–33.
Stein-Behrens, B.A., Saplosky, R.M., 1992. Stress, glucocorticoids, and aging. Aging Clin. Exp. Res. 4, 197–210.
Sterling, P., Eyer, J., 1988. Allostasis: a new paradigm to explain arousal pathology. In: Fisher, S., Reason, J. (Eds.), Handbook of Life Stress, Cognition and Health. Wiley, New York, pp. 629–649.
Stratholt, M.L., Donaldson, E.M., Liley, N.R., 1997. Stress induced elevation of plasma cortisol in adult female coho salmon (*Oncorhynchus kisutch*), is reflected in egg cortisol content, but does not appear to affect early development. Aquaculture 158, 141–153.
Sumpter, J.P., 1997. The endocrinology of stress. In: Iwama, G.W., Sumpter, J., Pickering, A.D., Schreck, C.B. (Eds.), Fish Stress and Health in Aquaculture. Cambridge Univ. Press, Cambridge, pp. 95–118.
Sumpter, J.P., Carragher, J.F., Pottinger, T.G., Pickering, A.D., 1987. Interaction of stress and reproduction in trout. In: Idler, D.R., Crim, L.W., Walsh, J.M. (Eds.), Proceedings of the 3rd International Symposium on Reproductive Physiology of Fish. St. John's, Newfoundland, pp. 299–302.
Takahashi, L.K., Turner, J.G., Kalin, N.H., 1998. Prolonged stress-induced elevation in plasma corticosterone during pregnancy in the rat: implications for prenatal stress studies. Psychoneuroendocrinology 23, 571–581.
Tatner, M.F., 1996. Natural changes in the immune system of fish. In: Iwama, G., Nakanishi, T. (Eds.), The Fish Immune System Organism, Pathogen, and Environment. Academic Press, San Diego, CA, pp. 255–287.

Tripp, R.A., Maule, A.G., Schreck, C.B., Kaattari, S.L., 1987. Cortisol-mediated suppression of salmonid lymphocyte responses in vitro. Dev. Comp. Immunol. 11, 565–576.

Truscott, B., 1979. Steroid metabolism in fish. Identification of steroid moieties of hydrolysable conjugates of cortisol in the bile of trout *Salmo gairdneri*. Gen. Comp. Endocrinol. 103, 301–307.

Van Der Kraak, G., Munkittrick, M.E., McMaster, M.E., Portt, C.B., Chang, J.P., 1992. Exposure of bleached kraft mill effluent disrupts the pituitary–gonadal axis of white sucker at multiple sites. Toxicol. Appl. Pharmacol. 115, 224–233.

Van Raaij, M.T.M., Pit, D.S.S., Balm, P.H.M., Steffens, A.B., van den Thillart, G.E.E.J.M., 1996. Behavioral strategy and the physiological stress response in rainbow trout exposed to severe hypoxia. Horm. Behav. 30, 85–92.

Van Tienhoven, A., 1983. Reproductive Physiology of Vertebrates. 2nd edn. Cornell Univ. Press, Ithaca, 491 pp.

Vijayan, M.M., Moon, T.W., 1994. The stress response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. Can. J. Zool. 72, 379–386.

Vijayan, M.M., Pereira, C., Moon, T.W., 1994. Hormonal stimulation of hepatocyte metabolism in rainbow trout following an acute handling stress. Comp. Biochem. Physiol. 116, 321–329.

Ware, D.M., 1984. Fitness of different reproductive strategies in teleost fishes. In: Potts, G.W., Wootton, R.J. (Eds.), Fish Reproduction: Strategies and Tactics. Academic Press, London, pp. 349–366.

Wendelaar-Bonga, S., 1997. The stress response in fish. Phys. Rev. 77, 591–625.

Wiegand, M.D., 1996. Composition, accumulation and utilization of yolk lipids in teleost fish. Rev. Fish. Biol. and Fish. 6, 259–286.

Yano, T., 1996. The nonspecific immune system: humoral defense. In: Iwama, G., Nakanishi, T. (Eds.), The Fish Immune System Organism, Pathogen, and Environment. Academic Press, San Diego, CA, pp. 106–157.

Yeoh, C.G., 1993. The effects of hormones on development of embryonic and post embryonic salmonids, and hormone metabolism during these stages. MS Thesis, Oregon State University, 120 pp.

Yeoh, C.-G., Schreck, C.B., Feist, G.W., Fitzpatrick, M.S., 1996a. Endogenous steroid metabolism is indicated by fluctuations of endogenous steroid and steroid glucorinide levels in early development of the steelhead trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 103, 107–114.

Yeoh, C.-G., Schreck, C.B., Fitzpatrick, M.S., Feist, G.W., 1996b. In vivo steroid metabolism in embryonic and newly hatched steelhead trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 102, 197–209.

Yousif, A.N., Albright, L.J., Evelyn, T.P.T., 1994a. In vitro evidence for the antibacterial role of lysozyme in salmonid eggs. Dis. Aquat. Org. 19, 15–19.

Yousif, A.N., Albright, L.J., Evelyn, T.P.T., 1994b. Purification and characterization of a galactose-specific lectin from the eggs of coho salmon, *Oncorhynchus kisutch*, and its interaction with bacterial fish pathogens. Dis. Aquat. Org. 20, 127–136.

Yousif, A.N., Albright, L.J., Evelyn, T.P.T., 1995. Interaction of coho salmon, *Oncorhynchus kisutch*, egg lectin with the fish pathogen *Aeromonas salmnicida*. Dis Aquat. Org. 21, 193–199.

Zohar, Y., 1980. Dorsal aorta catheterization in rainbow trout (*Salmo gairdneri*): I. Its validity in the study of blood gonadotropin patterns. Reprod. Nutr. Dev. 20, 1811–1823.