

# Hormones, behaviour and conservation of littoral fishes: current status and prospects for future research

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## WHY STUDY (LITTORAL) FISH?

Teleosts are the most diverse taxa of living vertebrates representing a very successful lineage of recently evolving organisms (Nelson, 1994). Bony fishes are good model systems for a comparative approach to the study of the relationship between hormones and social behaviour since they exhibit the widest range of reproductive behaviours and mating systems among vertebrates (reviewed by Demski, 1987). The diversity of described reproductive patterns includes:

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- (1) Gonochoristic species, composed of individuals with either testis or ovaries and that remain of the same gonadal sex all their lives.
- (2) Sequential hermaphroditic species, in which individuals at a certain stage of their life cycle change sex. In protogynous species individuals first develop and function as females and later change to males (common in wrasses, sea bass, groupers, and parrotfish; Warner, 1984). Conversely, in protandrous species individuals first mature as males and then change sex to females (occurs in some wrasses, porgies, damselfish and morey eels; Warner, 1984). The sex-change process may be elicited by several factors including relative body size, age, sex ratio in the population and social status (Shapiro, 1979; Warner, 1984; Grober, 1998). Serial hermaphroditism is a less common phenomenon. It has been described in the goby *Trimma okinawae* (Sunobe & Nakazono, 1993) and is characterised by repeated sex reversal by the same individual in response to social modifications.
- (3) Simultaneous hermaphroditic species, in which sexually mature individuals have functional testis and ovaries at the same time. Two different modes of reproduction can be found among simultaneous hermaphrodites: species in which individuals trade eggs and sperm reversing their roles sequentially during mating (e.g. several serranids of the genus *Serranus* and *Hypoplectrus*; Fischer, 1984), and species in which individuals self-fertilise themselves (e.g. the killifish *Rivulus marmoratus*; Harrington, 1961).
- (4) Parthenogenic species, in which all individuals have ovaries and reproduce asexually (e.g. the amazon molly *Poecilia formosa*; Hubbs & Hubbs, 1932; Schartl et al., 1995).

This wide variation in reproductive patterns makes teleost fishes a chosen group for the study of the proximate causes of sexual plasticity in vertebrates, namely the variation in mating systems, sex-change, and alternative reproductive tactics.

## RESEARCH QUESTIONS

*Mating systems and endocrine responsiveness*

Polygynous males should present higher levels of androgens for longer periods during the breeding season than monogamous males (i.e. males from monogamous or polyandrous species). This would reflect the fact that polygynous males do not participate in parental care of offspring and compete for access to mates during a longer period of time (Wingfield et al., 1990). In the only comparative study published so far to test this hypothesis, 20 species of Passeriformes, for which the breeding system and the androgen levels were known, were surveyed. Males of polyandrous and monogamous species showed a higher endocrine responsiveness (difference between breeding baseline and maximum response) to social interactions than males of polygynous species (Wingfield et al., 1990). This relationship between breeding systems, patterns of parental care and androgen levels were further stressed by the fact that androgen-implanted males of a monogamous species became polygynic and deserted their mates (Wingfield 1984).

Since teleost fishes display a large variation of breeding strategies and parental care types they are an ideal group of animals to conduct comparative studies of the association of mating systems with patterns of hormone-behaviour relationships. The plasticity of the mating systems is high and in a few species males may switch mating tactics (e.g. Saint Peter's fish, *Sarotherodon galilaeus*; Iles & Holden, 1969; Schwanck & Rana, 1991), allowing a test of the challenge hypothesis at the species level.

*Sex-change*

Although the mechanisms of sex-change have been the subject of intensive study (for a review see Lutnesky, 1994) the endocrine basis for sex-change is still controversial (see Grober, 1998). Early studies in

protogynous wrasses of the genus *Thalassoma* showed that injections of testosterone given to primary males and females could trigger the expression of the terminal phase male colouration and in some cases the development of male gonads in the females (Reinboth, 1975; Chan & Yeung, 1983).

On the other hand, more recent work in the stoplight parrotfish *Sparisoma viride* suggests that sex-change can be the cause, not the result, of changing levels of sex steroids during sex-change. In this species the increase in 11-ketotestosterone (11-KT) follows, not precedes, behavioural and colour sex-change (Cardwell & Liley, 1991b). Moreover, gonadectomized female bluehead wrasses (*Thalassoma bifasciatum*) when placed in a social group from which the terminal phase male was removed showed rapid behavioural sex-change (Godwin, 1996). Although in this study behavioural sex-change occurred in the absence of their main source of sex steroids, the role of adrenal steroids could not be ruled out. Further studies are needed to disentangle the relationship between sex steroids and behaviour in sex-changing teleosts.

#### *Alternative reproductive tactics*

In the last years evidence has accumulated for the occurrence of alternative reproductive tactics (ART) in different fish families – Salmonidae, Cyprinidae, Gasterosteidae, Cyprinodontidae, Poeciliidae, Centrarchidae, Cichlidae, Pomacentridae, Labridae, Gobiidae, Blenniidae, among many others (for a review see Taborsky, 1994). These ART occur, in general, in species in which males compete for access to mating territories and for female attraction. Smaller males, with a lower competitive ability, may adopt one of two ART: to act as sneakers – interacting with the dominant male-female pair during spawning and achieving the fertilisation of part of the clutch; or to act as satellites – being tolerated by the resident male and participating, in some cases, in nest defence (Taborsky, 1994).

The ART may be characterised by a precocious reproductive strategy in comparison to the dominant males that reach sexual maturity much later during their life. However, both tactics may reach equivalent results in terms of fitness, e.g., *Lepomis* and *Oncorhynchus* (Gross, 1984). On the other hand, in some species younger males may adopt a sneaker tactic until they are big enough to become territorial, e.g. *Pomatochistus microps* (Magnhagen, 1992). Despite having mature gonads, males with ART fail to reveal any of the secondary sex characters typical of the dominant males of the species. In general, these alternative males possess much higher gonadosomatic indexes than territorial males, possibly due to the fact that they have restricted access to females and fewer opportunities for fertilisation (Taborsky, 1994).

From the point of view of proximate causes, ART are interesting models to study the uncoupling of different aspects of male reproductive biology, namely gonad maturation, expression of secondary sex characters, and activation of male sexual behaviour.

In a recent review of the hormonal basis of male sexual dimorphism in teleosts, Brantley et al. (1993a) came to the following conclusions:

- I) Androgen profiles vary according to male mating tactics;
- II) Courting males (i.e. territorial, nest-holders, etc. according to the specific mating systems of the different species) consistently have higher levels of 11-KT, but not of testosterone, than non-courting males (i.e. sneakers or satellites).

This pattern is present both in fixed (e.g. *Porichthys notatus* and *Lepomis macrochirus*) or sequential (e.g. *Salmo salar*, *Sparisoma viride*, *Thalassoma duperrey* and *Coris julis*) alternative tactics. These endocrine differences may reflect a role for androgens in the differences observed between male morphs in terms of:

- I) Reproductive behaviour;
- II) Differentiation of secondary sex characters;
- III) Relative size of the testis.

- I) *Androgens and reproductive behaviour* – The hormonal differences detected between the two male types can be interpreted either as the causes or as the effects of the behavioural differences. As 11-KT is the androgen that best reflects the behavioural differences between the two male types it is expected to play a role in the activation of reproductive behaviour, namely in the establishment of a reproductive territory and in courtship. Although there is a large body of literature on the role of androgens on the expression of reproductive behaviours (for a review see Liley & Stacey, 1983) there are few studies that have tested the role of 11-KT. Borg (1987) implanted 11-ketoandrostenedione (a precursor of 11-KT) to castrated males of three-spined stickleback (*Gasterosteus aculeatus*) and found that this treatment was effective in recovering the different aspects of male reproductive behaviour (i.e. territoriality, nest construction, and courtship). Kindler et al. (1991) also implanted 11-KT and testosterone in the sunfish (*Lepomis macrochirus*) and found that 11-KT was more effective than testosterone both in a pre-spawning behaviour (rim-circling) and in parental care (anti-predator-defence).

Studies in the natural habitats of seasonal reproducing species show that the levels of circulating 11-KT rise during the phase of establishment of a territory, when agonistic interactions are more intense (*Lepomis macrochirus*, Kindler et al., 1989; *Hypsypops rubicundus*, Sikkell, 1993; *Chromis dispilus*, Pankhurst, 1990). 11-KT levels also rise in terminal phase males of stoplight parrotfish (*Sparisoma viride*) when subject to experimental territorial intrusions and are correlated with territorial density and the frequency of agonistic interactions in the demoiselle (*Chromis dispilus*) (Pankhurst & Barnett, 1993). In *Oreochromis mossambicus* and in *Oncorhynchus mykiss* dominant males have higher levels of both 11-KT and testosterone, although the differences are larger in the first case (Liley & Kroon, 1995; Oliveira et al., 1996).

II) *Androgens and secondary sex characters* – Due to the above-mentioned relationship between 11-KT, male type and the expression of secondary sex characters an important role for this hormone in the differentiation of sexually dimorphic traits is to be expected. In fact, there is a large body of literature showing that most secondary sexual characters are androgen dependent, and that 11-KT is the most potent androgen in inducing their differentiation – sonic motor system in *Porichthys notatus* (Brantley et al., 1993b), median fins in *Betta splendens* (Leitz, 1987), gonopodium differentiation in Poecillids (Liley & Stacey, 1983). It should also be mentioned that in teleosts testosterone is also present in females, most times at similar or higher levels than in males, while 11-KT is only detected in males. Based on this evidence some authors propose that testosterone should be seen as a pro-hormone that can be metabolised into 11-KT in males and into estradiol-17 $\beta$  in females (Brantley et al., 1993a). Supporting 11-KT in the differentiation of secondary sex characters is also the fact that, in species without sexual dimorphism, males have low levels of the hormone (e.g. *Sardinops melanosticus*, Matsuyama et al., 1991; *Syngnathus typhle*, Mayer et al., 1991).

III) *Androgens and gonadal differences* – Levels of androgens, and of 11-KT, in particular, correlate in many species to GSI, levels being highest in pre-spawning/pre-milt producing fish (*Clupea harengus pallasi*; Carolsfeld et al., 1996; *Catostomus commersoni*; Scott et al., 1984). There is evidence for an association of levels with spawning behaviour but not spermiation (Barnett & Pankhurst, 1994). Furthermore, there is no evidence that milt production, its volume and density or the ionic composition of the seminal plasma could be related to the levels of 11-KT or testosterone (Baynes & Scott, 1985). Neither do the androgens appear to be very effective in inducing spermiation, unlike 1720 $\beta$ -P (Ueda et al., 1985). However, 11-KT appears to be a mediator of spermatogenesis (Miura et al., 1991) being synthesised under gonadotrophin stimulation by the somatic Leydig cells (Barry et al., 1989; Miura et al., 1991). In *Thalassoma duperrey*, a species with males showing alternative tactics, the

smaller initial-phase (IP) and group spawner males have large testis and high sperm production in comparison with larger, territorial, terminal-phase (TP) males. These males have much smaller testes than do IP males, but steroid-producing Leydig cells in the gonads of TP males appear more numerous and better developed. Testes of TP males produce more testosterone and especially 11-KT than do testes of IP males, and the production is more responsive to gonadotropin (Hourigan et al., 1991). The Leydig cell development and high levels of 11-KT production to the terminal male phenotype were more related to reproductive or aggressive behavior, rather than to male gametogenesis *per se* (Hourigan et al., 1991). In conclusion, differences in levels of androgens, in particular 11-KT, seem better explained by a role in modulating sexual and aggressive behaviour than by a physiological role in spermiation.

#### *Endocrine cycles and parental care*

It has been proposed the occurrence of a trade-off between androgens and paternal care. As levels of androgens increase above a breeding baseline the expression of parental care decreases. In many monogamous species with male parental care the experimental induction of higher levels of testosterone in parental males suppresses paternal behaviour and increases aggression (Silverin, 1980; Hegner & Wingfield, 1987). Several studies on the temporal variation in seasonal breeding birds and teleosts, show that androgen levels are higher in the beginning of the breeding season when territories are being established and when courtship is more intense, than in the following parental care phase (Pankhurst, 1990; Wingfield et al., 1987; Sikkel, 1993; see Table 1 for data on teleost fishes). However, in teleost species, in which mating and parental phases are not separated in time and males simultaneously guard multiple clutches of eggs at different developmental stages, as is the case of littoral fishes, the pattern of temporal androgen variation can be less marked or even absent (e.g. plainfin midshipman, Knapp et al., 1999; peacock blenny, Oliveira et al., in preparation). This is suggestive that the trade off between androgens

and the expression of male parental behaviours in species with paternal care, described for vertebrate species with dissociated mating and parental phases, may not be the rule for species in which mating and parental care widely overlap.

Table 1

*Variation in circulating androgen levels during the breeding season in male teleosts that display paternal care of offspring: M=mating phase; P=parental phase*

Species (Family)	Phase	Testosterone (ng/ml)	11-KT (ng/ml)	Author
<i>Syngnathus acus</i> (Syngnathidae)	M	15.2	3.6	Mayer et al., 1993
	P	6.4	0.9	
<i>Syngnathus typhle</i> (Syngnathidae)	M	3.2	2.4	Mayer et al., 1993
	P	2.0	0.9	
<i>Lepomis macrochirus</i> (Centrarchidae)	M	24	55 <sup>a</sup>	Kindler et al., 1989
	P	7	14 <sup>a</sup>	
<i>Chromis dispilus</i> (Pomacentridae)	M	4-6; 9 <sup>b</sup>	49 <sup>b</sup>	Pankhurst, 1990; Barnett & Pankhurst, 1994
	P	<1; 1.5 <sup>b</sup>	8 <sup>b</sup>	
<i>Hypsypops rubicundus</i> (Pomacentridae)	M	15 <sup>b</sup>	22 <sup>b</sup>	Sikkel, 1993
	P	9 <sup>b</sup>	7 <sup>b</sup>	

Note. (a) Values obtained one day after spawning; (b) Values extrapolated from published graphs.

#### METHODOLOGICAL AND LOGISTIC CONSTRAINTS

There are a number of technical difficulties in measuring hormones in teleost fishes. The first is related to body size. Most teleosts are small animals with a small volume of blood making blood sampling difficult without sacrificing the animal (e.g. most blennies and gobies). Conversely, other species may attain very large dimensions making handling difficult (e.g. parrotfishes).

A second difficulty is the fact that most steroid assays have been developed for mammalian steroids and teleosts produce a variety of non-classic steroids for most of which no assays are available.

The third difficulty consists of the water environment in which fish live. Human observers can only study fish in their natural habitat using scuba gear to a depth of approximately 40 meters. Below this depth the logistics involved in the study are complicated and require the use of submersibles or of remote operated vehicles. Even when studies are restricted to the littoral fishes (i.e. occurring to a depth of no more than 30 meters) the logistics involved in underwater research are still reasonably complicated. The time a diver can remain underwater varies with depth. For example, according to the diving security curve at a depth of 40m the diver can spend only 10 minutes.

In temperate waters temperature is an additional factor limiting research activities. Water turbulence can also be very accentuated in exposed rocky shores, which are the natural habitats of many littoral species such as blennies, gobies and gobiesocids. In summary, in temperate waters *in situ* research in fish behavioural endocrinology is both limited by depth, temperature and sea conditions. Finally working underwater (i.e. catching specific individuals, marking them and collecting blood samples) requires special training. Nevertheless, there are a few cases in which underwater blood sampling has been carried out successfully as was the case of the study in the demoiselle (*Chromis dispilus*) by Pankhurst (1990) .

#### NON-INTRUSIVE TECHNIQUES TO ASSAY STEROID HORMONES IN FISH

As we have discussed above, one of the constraints in the study of hormones and behaviour in littoral fish is the small body size of some species which limits classic blood sampling procedures. Moreover, blood collection techniques require capture, handling and intrusive manipulation of individuals, all of which elicit acute stress responses characterised by a

rise in plasma cortisol levels and other physiological changes (e.g. Strange et al., 1977; Barton et al., 1980; Pankhurst & Dedual, 1994; Pottinger et al., 1996; Pottinger, 1998; Pottinger et al., 1999; for a review see Barton, 1997). Based on the observation that large quantities of sex steroids and corticosteroids are released in large quantities through the urine, gills and intestine into the water (Canario & Scott, 1989; Scott & Liley, 1994; Scott & Sorensen, 1994; Vermeirssen & Scott, 1996), where they can be easily detectable, we have developed two specific protocols to assay steroid hormones, including cortisol, to minimise the problem of manipulation.

#### *Sampling steroid hormones in small fish*

Individual fish are placed in a container with a constant volume of water for a standard period of time. The products of its metabolism will be excreted into the water and will accumulate in it. Fish holding water is passed through a solid-phase C18 extraction column where the steroids will be adsorbed. Ethanol is used to elute the steroids from the column. Since steroids are mainly excreted in a conjugated form, either as sulphates or glucuronides, they are hydrolysed and extracted in succession, according to the protocol described in Figure 1, before radiimmunoassay.

The definition of the time the fish will be kept constrained in the jar and the amount of water used are two critical factors in this protocol that should be decided carefully. The confinement time should be kept to a minimum since it induces acute stress responses (e.g. Strange et al., 1978; Pankhurst & Sharples, 1992; for a review see Barton, 1997) but it should be enough for fish to release enough steroids in the water for detection by the radioimmunoassay. An additional problem that should be kept in mind is that a diffusional equilibrium between circulating steroids and water born steroids can be established at the gill surfaces (Vermeirssen & Scott, 1996). The volume of water used should also be kept to a minimum since passing large amounts of water through the solid phase extraction columns is time consuming and can be a limiting step in the set-up.

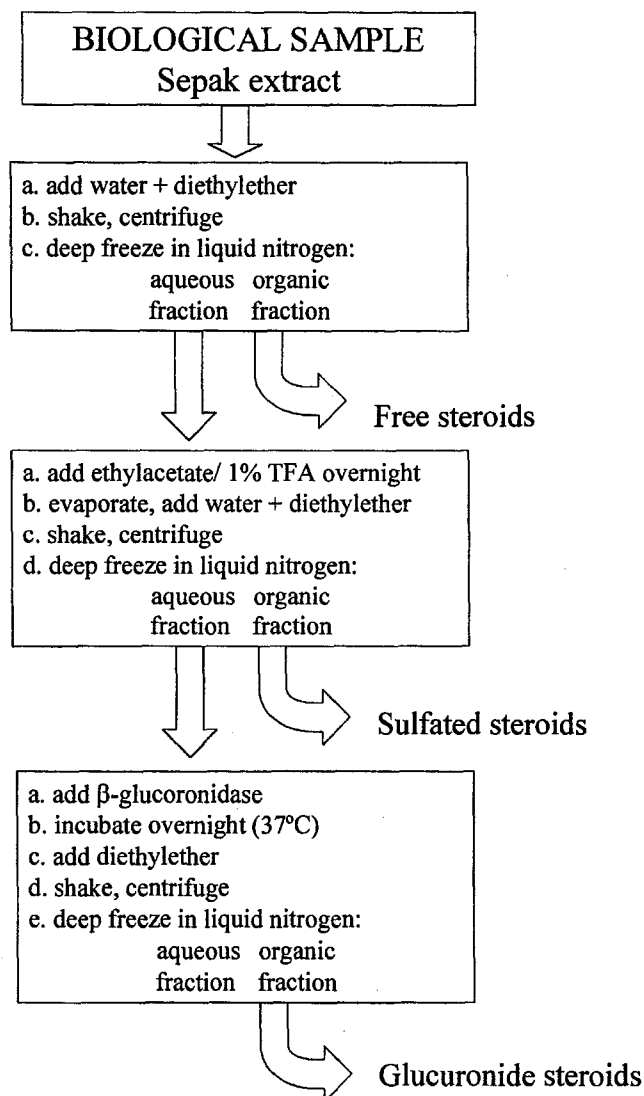


Figure 1. Extraction of different phases for the assay of steroids and steroid conjugates (for details see text).

Although we are still working on the validation of this technique, preliminary studies provide data with biological meaning.

In some blenniid species, males have an anal gland that secretes a female attractant sex pheromone (Laumen et al., 1974; Zeeck & Ide, 1996; Singh, 1989). It has recently been shown that one major product of the steroid metabolism in this gland is the progestin 17,20  $\beta$ -dihydroxy-4-pregnen-3-one (17,20  $\beta$ -P; Barata et al., 1998). We have placed male peacock blennies (*Salaria pavo*) in 0.5 l jars for 1.5 hr, and assayed several sex steroids from the water (see Figure 2A). The results clearly show that 17,20  $\beta$ -P is released in high concentrations. In another closely related species, the rock pool blenny (*Parablennius sanguinolentus*), a diandric species with nest-holder males with the full set of male sex characters and with satellite males lacking the pheromone producing anal gland, we have collected samples of 0.33 l of water in tide pools at the entrance of nests and in other places far from nest sites (control). We have also sampled holding water from the two male types. The results presented in Figure 2B and Figure 2C suggest that nest-holders are releasing more 17,20  $\beta$ -P into the water and that decreasing concentrations of 17,20  $\beta$ -P with distance from the entrance of nests in tide pools can be assayed.

These two observations although preliminary suggest that the methodology can produce meaningful data from a biological point of view. Nevertheless, more work is required for its validation.

#### *Non-invasive sampling of steroid hormones in unrestrained fish*

The assay of steroid levels from unrestrained animals have been used for different purposes (e.g. monitoring animal welfare in animal farming or in zoos) for a long time. Recently the development of non-invasive procedures to replace blood sampling have been using different biological fluids ranging from saliva from zoo and farm animals (Cook et al., 1996) to urine or faeces in wild populations of birds (Bercovitz et al., 1982;

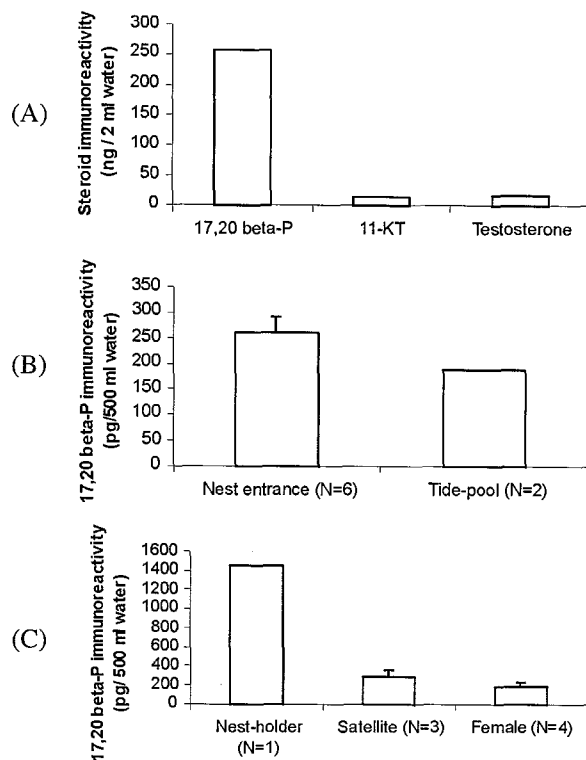


Figure 2. Levels of sex steroids measured from water: (A) 17, 20  $\beta$ -P, 11-KT and testosterone concentrations in holding water from *S. pavo* breeding males; (B) 17, 20  $\beta$ -P concentrations in water collected from tide pools in which *P. sanguinolentus* nests, at different distances from nests; (C) 17, 20  $\beta$ -P concentrations in holding water from different *P. sanguinolentus* sexual morphotypes

Kotrschal et al., 1998) and mammals (Möstl et al., 1984; Miller et al., 1991; Anzenberger & Gossweiler, 1993; Jurke et al., 1994; Graham & Brown, 1996). Nevertheless, the use of non-invasive methods to assay steroids from fish wild populations have never been reported to our knowledge. A major difficulty is that conjugated steroids may easily be

released from the faeces into the water, and in the case of urine it dissolves in the surrounding water and excreted steroids will be quickly dispersed in the environment. One possibility is to use fresh fish faeces and to isolate them from the water as soon as possible. Such approach can require following focal individuals while diving or snorkelling and wait until they defecate. At this stage a quick intervention is essential to quickly catch the faeces and place them in a sealed tube. After collection, the faeces can be stored in a freezer for months (-20°C) until they are extracted using butanol, and the steroids assayed using standard techniques (e.g. RIA). We have recently applied this approach to measure cortisol levels in reef fishes of the Red Sea in two different locations exposed to different levels of anthropogenic stressors (see below).

#### ENDOCRINOLOGY AND CONSERVATION BIOLOGY

Wingfield et al. (1997) recently emphasised the important role of endocrinology for conservation biology. Namely by increasing our knowledge of the reproductive biology of endangered species, by helping to control the reproduction of exotic introduced species, by monitoring environmental estrogens that may disrupt the breeding of natural populations, by allowing the assessment of stress in wild individuals, and by monitoring animals that have been reintroduced to natural conditions in captive breeding programs. Although all these ways in which hormone studies may contribute to conservation programs deserve attention in the present paper we will address only two: the monitoring of environmental steroids and their potential disruptive effect in reproductive function and the assessment of stress in free-living animals.

##### *Effects of environmental steroids: estrogens and androgens in the wild*

In recent years there has been an increasing awareness of the potential effects of environmental contaminants on the disruption of the endocrine

system with detrimental consequences for the reproduction of wildlife and human populations (Colborn et al., 1993). The increasing concentrations of phyto-sterols and other endocrine disrupting chemicals in freshwaters in industrialized countries has been suggested to be potentially related to a decrease in sperm counts in male humans and to an increased incidence of breast cancer in women (Colborn et al., 1993; Safe, 1995).

The most famous example on the disruption of reproduction by environmental steroids comes from mosquitofish exposed to paper mills discharging kraft-mill effluents which contain phytosterols (Howell et al., 1980; Bortone et al., 1989). It was observed that some females occurring downstream of the effluent exhibited signs of masculinisation such as a variable differentiation of the anal fin into a gonopodium. In the upstream population no masculinised females were found. A more detailed analysis also revealed that masculinised females exhibited some elements of male behaviour. On the other hand, males from the downstream population showed a precocious expression of secondary sex characters and reproductive behaviour. Thus, the incidence of female mosquitofish masculinization in natural populations can be used as a bioassay for the monitoring of androgenic compounds in freshwaters.

More recently reproductive physiological tests using fish have been developed to assess the impact of endocrine disruption chemicals in vertebrate reproduction. For example the measurement of circulating sex steroids have been included in many studies of wild fish populations. It has been shown that fish populations living downstream of kraft pulp mill effluents have lower plasma levels of sex steroids which were correlated with other reproductive indexes such as gonad size and the expression of secondary sex characters (Munkittrick et al., 1992; van der Kraak et al., 1992). These results suggest a requirement for the integration of fish whole organism in *vivo* or laboratory in *vitro* screening tests in studies assessing environmental disruptive chemicals in aquatic habitats (Van der Kraak et al., 1995).

### *Stress in the wild*

There has been an increasing interest in the measurement of stress physiological indices in free-living animals as indicators of environmental degradation and anthropogenic stressors (Wingfield et al., 1997; Hofer & East, 1998). This approach assumes stress has important implications for the fitness and/or the reproductive success of the individuals and consequently for the persistence of animal populations in the wild (Hofer & East, 1998). Different stress physiological indicators have been used ranging from measurement of the circulating levels of glucocorticosteroids as indicators of the adrenocortical response (i.e. cortisol in humans, some mammals and teleost fish and corticosterone in some other mammals, amphibians, reptiles and birds; Wingfield et al., 1997), heart rate, reproductive suppression, torpor, etc (Hofer & East, 1998). For example urine collected from the snow has been used to assess physiological condition of free-living wolves and deer (Mech et al., 1987; Delgiudice et al., 1989).

Dunlap and Wingfield (1995) surveyed baseline levels of plasma corticosterone and the adrenal responsiveness in 6 populations of the fence lizard (*Sceloporus occidentalis*) over its geographical distribution range in North America. They found that adrenocortical responsiveness to handling stress was higher in peripheral populations than in central ones. These observations have obvious implications for conservation since it suggests that habitat fragmentation would make the population more exposed to detrimental effects of stress.

In another study the effects of an oil spill off the coast of Patagonia (Argentina) on breeding magellanic penguins (*Spheniscus magellanicus*) was described. Both male and female oiled penguins had lower levels of circulating sex steroids and were not seen in the breeding colony (Fowler et al., 1995). Moreover, few pairs with oiled partners established territories and laid eggs. Interestingly, only oiled females had significantly higher circulating levels of corticosterone.

In teleost fishes, studies of stress in wild populations have been carried out, mainly in salmonid species (e.g. Pickering & Pottinger, 1987). Recently, stress levels of free-living populations of non-salmonid species, mainly from freshwater habitats, have started to be used as early indicators of environmental degradation. For example, circulating levels of cortisol in yellow perch (*Perca flavescens*) have been used as indicators of heavy metal contamination in lakes (Brodeur et al., 1997).

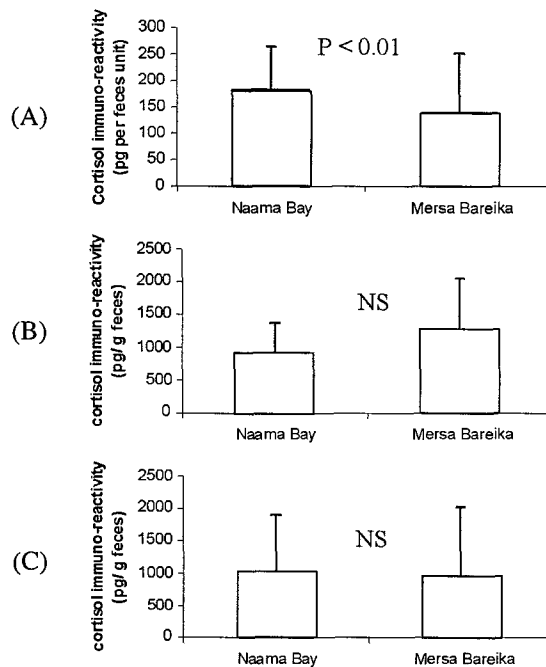
#### A CASE STUDY:

#### STRESS LEVELS IN FISH EXPOSED TO ECO-TOURISM IN THE RED SEA

Ecotourism, that is, the recreational activities involving non-consumptive wildlife utilisation, has been recommended as a measure in wildlife conservation since it represents a major income source for some regions (Goodwin, 1996). With a few exceptions (tourism and nesting turtles: Jacobson & Lopez, 1994; Antarctic eco-tourism and breeding penguins: Culik & Wilson, 1995; whale watching: Evans, 1996) the impact of ecotourism in protected areas has been neglected and whether it really contributes for the protection of habitats/species is a matter of debate (Duffus & Dearden, 1990; King & Stewart, 1996). Thus, further studies on the effect of tourism related stressors are required and the evaluation of their impact on wildlife needs to be assessed.

The Red Sea is a major touristic destination. Each year millions of tourists converge to the Red Sea to explore the vast coral reefs in warm waters with an excellent visibility. The impact of human activities (scuba-diving, snorkelling, underwater noise from motor boats, sewage, etc) on the coral reef communities has been studied, and protected areas with restricted access have been created (e.g. Fishelson, 1999). The assessment of stress levels in wild animals may be used as an early indicator of the impact of human activities (anthropogenic stress) on the ecosystem and thus as bio-

indicators of sustainable levels of touristic exploitation for a given area. We have assessed stress levels in reef fish populations at two locations in Sharm el Sheik area (Egypt): Naama Bay, a major tourist spot highly exposed to anthropogenic stressors, and Mersa Bareika, an area of the Ras Mohammed National Park with restricted access (a special permit is required for diving in this area). Cortisol levels were measured from extracts of faeces by radioimmunoassay. Faecal samples were collected by snorkelling and following a fish until it defecated. Faeces were collected with a plastic nipper and placed in a tube. The tubes were stored at -20°C until the samples were processed. Free and conjugated steroids were extracted with butanol followed by hydrolysis and extraction separately of free, sulphated and glucuronidated steroid (Scott & Vermeirssen, 1994). Three species were selected based on the consistency of their faeces which influenced the feasibility of the faecal collection: two parrotfishes (*Scarus niger* and *S. ferrugineus*) and one surgeonfish (*Ctenochaetus striatus*). A summary of the the results is presented in Figure 3. Cortisol levels are significantly higher in surgeonfish from the exposed location than in the protected area (Figure 3A). However, no significant differences were found between the two areas concerning the cortisol levels in the two parrotfish species studied (Figure 3B and Figure 3C). These results may reflect different ways of coping with stress between surgeonfishes and parrotfishes. Indeed, parrotfishes approach divers and seldom come to feed by hand, while surgeonfishes are more furtive and avoid divers. Thus, the effects of anthropogenic stress may vary from species to species and the choice of a target species is an important step in impact studies, since different species may show different reactions or even a reaction in the same direction but with different thresholds. These preliminary results open the way to the potential use of fish stress hormone levels from faecal samples as a bio-assay for anthropogenic stressors in littoral environments.



*Figure 3.* Cortisol concentrations in faeces collected from free-living reef fishes from the Red Sea in two different locations, Naama Bay (exploited area) and Mersa Bareika (nature reserve): (A) Lined bristletooth, *Ctenochaetus striatus*, Acanthuridae; (B) Swarthy parrotfish males, *Scarus niger*, Scaridae; (C) Rusty parrotfish males, *Scarus ferrugineus*, Scaridae.

#### ACKNOWLEDGEMENTS

The authors would like to thank Luis Carneiro and Elsa Couto for invaluable assistance in laboratory work and the Max-Planck Institut für Verhaltenphysiologie and Deutsche Forschungsgemeinschaft (R.B.) and Fundação para a Ciência e Tecnologia (R.O.) (UI&D 331/94 and grant PRAXIS/PCNA/C/BIA/94/96) for financial support.

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