also in part by a factor that is derived from the host from which the inoculum is prepared. For this reason, we feel that it is important for investigators to reexamine primate susceptibility to the scrapie agent as it exists in sheep tissues.

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## Temperature Tolerances of Some Closely Related Tropical Atlantic and Pacific Fish Species

Abstract. *Species of Pacific shallow-water fish are more tolerant of low temperatures than Atlantic species are. At high temperatures Atlantic species are more tolerant than Pacific species. For species pairs of* Bathygobius *differences in the tolerance of low temperatures are small and can be removed by acclimation to 23°C. Differences in the tolerance to low temperature in transisthmian species of* Apogon, *however, are large and persist after acclimation to 23°C. Some Pacific species adapt to the cooler temperatures of their habitat through increasing their rates of oxygen consumption at ambient temperatures or decreasing the dependence of oxygen uptake rate on temperature, or both.*

Atlantic and Pacific Panamanian shore fishes are useful for comparative studies of thermal tolerance because many species of the two oceans are closely related and the temperatures of the two oceans differ slightly. Phyletic affinities of the two faunas stem from late Pliocene times when, prior to the emergence of the Central American Isthmus, the Neotropical sea fauna was continuous (*1*). Physical oceanographic differences in the two oceans have been thoroughly studied; the eastern tropical Pacific has a lower average and more variable temperature as well as a more variable salinity than the tropical western Atlantic (*2*). Comparative studies of Atlantic and Pacific species make possible the determination of the effect of post-Pliocene differences in temperature on the temperature adaptations of particular species, and the relationship between thermal tolerance and taxonomic affinity in particular species pairs. Interest in comparative studies is further generated by the proposal for a Central American sea-level canal and by the need for assessing the colonizing capacities of different species (*3*).

As indices of temperature sensitivity, critical thermal (CT) maxima and minima were determined for three Atlantic and Pacific species pairs: the damselfishes (family Pomacentridae) *Abudefduf saxatilis* (Atlantic) and *Abudefduf troschelii* (Pacific), the cardinalfishes (Apogonidae) *Apogon maculatus* (Atlantic) and *Apogon dovii* (Pacific), and the gobies (Gobiidae) *Bathygobius soporator* (Atlantic) and *B. ramosus* (Pacific) (*4*). Critical thermal minima only were determined for a fourth species pair of soapfishes (Grammistidae) *Rypticus subbifrenatus* (Atlantic) and *R. nigripinnis* (Pacific). For determination of CT maxima and minima freshly collected groups of fish (of similar size) were heated or cooled from ambient seawater temperatures (26° to 28°C) in a filtered, aerated aquarium until they died. Rates of heating and cooling were regulated as precisely as possible and varied from 1.6° to 2.3°C per hour for heating and from 1.0° to 1.4°C per hour for cooling. In an additional experiment, species pairs of *Apogon* and *Bathygobius* were acclimated to 23°C for 15 days before determination of CT minima. Acclimation in the laboratory removes the effects of environmental variability and permits the determination of an organism's genetic capacity for thermal adaptation (*5*).

For all four species pairs, the Pacific species have significantly lower CT minima (Table 1) (*6*). The CT maxima of Atlantic fishes are significantly higher than those of the Pacific fishes (Table 1). The CT minima of the four species that had been acclimated to 23°C are significantly lower than those for unacclimated fishes (Table 2). Comparisons of Atlantic and Pacific fishes within this acclimated group show that *Apogon dovii* has a greater tolerance to cold than *Apogon maculatus* but that the CT minima of *B. ramosus* and *B. soporator* are not significantly different (Table 2).

The rate of oxygen uptake as a function of temperature was determined for three Atlantic and Pacific species pairs (*7*). Regression equations relating total oxygen consumption and wet body weight were formulated for each species at each temperature. These sets of equations were tested for interspecific differences in slope and position by F ratios. Regression line values of oxygen uptake from 15° to 35°C for 5-g individuals of *B. ramosus, B. soporator, Apogon dovii,* and *Apogon maculatus* are given (Table 3). Values for *R. nigripinnis* and *R. subbifrenatus* are line values for the regression of temperature on oxygen consumption for specimens in the size range of 0.9 to 3.4 g (Table 3). No measurements were made for *Abudefduf troschelii* and *Abudefduf saxatilis.*

From 20° to 35°C the rate of oxygen uptake of *Apogon dovii* is significantly

Table 1. Critical thermal maxima and minima of Atlantic (A) and Pacific (P) fishes. Values are mean, 95 percent confidence intervals of the mean, and sample sizes (in parentheses).

| Species | Ocean | Mean temperature (°C) |
|---|---|---|
| *Critical thermal maxima* | | |
| *Apogon dovii* | P | 37.23 ± .05 (12) |
| *Apogon maculatus* | A | 37.61 ± .26 (10) |
| *Abudefduf troschelii* | P | 38.67 ± .19 ( 8) |
| *Abudefduf saxatilis* | A | 39.30 ± .14 (13) |
| *Bathygobius ramosus* | P | 40.70 ± .04 (21) |
| *Bathygobius soporator* | A | 40.85 ± .08 (20) |
| *Critical thermal minima* | | |
| *Apogon dovii* | P | 10.52 ± .23 (20) |
| *Apogon maculatus* | A | 13.00 ± .31 (11) |
| *Abudefduf troschelii* | P | 11.15 ± .29 (10) |
| *Abudefduf saxatilis* | A | 11.58 ± .20 (15) |
| *Rypticus nigripinnis* | P | 8.60 ± .48 (16) |
| *Rypticus subbifrenatus* | A | 10.20 ± 1.02 ( 6) |
| *Bathygobius ramosus* | P | 9.49 ± .13 (20) |
| *Bathygobius soporator* | A | 10.07 ± .17 (20) |

Table 2. Critical thermal minima of Atlantic (A) and Pacific (P) fishes following acclimation to 23°C. Values are mean, 95 percent confidence intervals of the mean, and sample sizes (in parentheses).

| Species | Ocean | Mean temperature (°C) |
|---|---|---|
| *Apogon dovii* | P | 8.12 ± .32 (10) |
| *Apogon maculatus* | A | 9.14 ± .18 (13) |
| *Bathygobius ramosus* | P | 8.39 ± .47 (11) |
| *Bathygobius soporator* | A | 8.05 ± .54 (10) |

higher than that of *Apogon maculatus*. The rates of these species are not significantly different at 15°C. When the decrease in the rate of oxygen uptake from 35° to 20°C is considered, the rate of *Apogon maculatus* at 15°C is higher than expected and is apparently caused by the loss of equilibrium and associated excessive fin motion of a righting response of some individuals tested. Specimens of *Apogon dovii* never lost equilibrium at 15°C. Although the rate for *B. soporator* is displaced lower with cooling, no significant differences are found in comparison with *B. ramosus*. High variability of rates of oxygen uptake at low temperatures has been reported by other investigators (*8*) and may serve to obscure differences between *B. ramosus* and *B. soporator*. The regressions of temperature on oxygen consumption for groups of *R. subbifrenatus* and *R. nigripinnis* of similar size are significantly different in slope and position. *Rypticus nigripinnis* has a higher rate at 30°C, and its slope from 15° to 35°C is less.

*Bathygobius ramosus* and *B. soporator* are closely related and ecologically similar; $F_1$ progeny have resulted from interspecific crosses (*9*), and both species have similar latitudinal and vertical distributions. The predominant occurrence of both species in the high littoral zone makes the overall range of habitat temperatures to which they are exposed very similar.

From these studies on temperature physiology it appears that there has been no selection in *B. ramosus* and *B. soporator* in response to temperature differences in the habitats. This is indicated by similar CT minima after acclimation to 23°C and similar rates of oxygen uptake from 15° to 35°C. The zones of thermal tolerance (number of degrees separating the CT maxima and minima) provide an additional index to the thermal adaptive state of an organism (*10*) and are similar for these two species—31.30°C for *B. ramosus* and 31.15°C for *B. soporator*. It is probably true that no selection as a result of difference in habitat has occurred in *Abudefduf saxatilis* and *Abudefduf troschelii*, which have comparable zones of tolerance (27.70° and 27.55°C), are practically indiscernible morphologically, and have similar habitat preferences (*11*). Small-scale differences in the thermal tolerances of transisthmian species of *Bathygobius* and *Abudefduf*, as indicated by the displacement of the tolerance zones of Pacific species in the direction of cooler water, support the thesis that although underlying genetic capabilities for temperature adaptation are the same, differences in environmental temperatures can act to modify the adaptive state.

The species of *Bathygobius* and *Abudefduf* studied in this work are morphologically and ecologically more similar than are the species of *Apogon* and *Rypticus*. *Apogon dovii* is structurally similar to *Apogon maculatus* although it attains a larger size. The species are more divergent ecologically; *Apogon maculatus* lives sympatrically with other species of *Apogon* and has a wider latitudinal and bathymetric distribution than *Apogon dovii*. *Rypticus nigripinnis* occurs on rock and mud substrate in littoral and sublittoral localities, whereas *R. subbifrenatus* occurs only on coral reefs. The greater distance of the phyletic relationship in these species pairs is reflected in the magnitude of the displacement of the CT minima of the Pacific species, 1.60° lower for *R. nigripinnis* and 2.48°C lower for *Apogon dovii* (Table 1). The zone of tolerance of *Apogon dovii* (26.70°C) is significantly larger than that of *Apogon maculatus* (24.60°C), and differences in the temperature sensitivities of these species are apparent after acclimation to 23°C. Both higher rates of oxygen uptake in the two Pacific species at ambient temperatures and the lower slope of the rate-temperature function for *R. nigripinnis* are typical adaptations made by poikilotherms for cooler or more variable temperatures (*5, 12*). These data show that the differences in temperature sensitivity of *Apogon dovii* and *Apogon maculatus* have a genetic basis, perhaps reflecting differences in habitat temperature since Pliocene times. This is probably true for *R. nigripinnis* and *R. subbifrenatus* and is expected on the basis of taxonomic and ecological differences between these species.

Predictions about the ecological success of fishes that transit the proposed sea-level canal from either ocean must be based on a variety of comparative studies. The upper and lower lethal temperatures of the species studied are well beyond the thermal extremes found in the two oceans. For this reason thermal differences between the two oceans will not constitute a barrier to the transisthmian dispersal of adult fishes. However, final judgment on this question must await further studies on the thermal tolerances of juvenile fishes, and on the effects of

Table 3. Rates of oxygen consumption with 95 percent confidence intervals of Atlantic (A) and Pacific (P) fishes. Values for species of *Bathygobius* and *Apogon* are rates of 5-g individuals taken from regressions relating weight and rate of oxygen consumption for each temperature. Values for species of *Rypticus* are from regressions relating rate of oxygen consumption and temperature for similarly sized fish.

| Species | Ocean | Oxygen consumption (ml $kg^{-1}$ $hour^{-1}$) at | | | | |
|---|---|---|---|---|---|---|
| | | 15°C | 20°C | 25°C | 30°C | 35°C |
| *Apogon dovii* | P | 61 ± 9 | 100 ± 11 | 134 ± 12 | 200 ± 8 | 280 ± 2 |
| *Apogon maculatus* | A | 46 ± 8 | 60 ± 8 | 85 ± 14 | 116 ± 8 | 168 ± 50 |
| *Bathygobius ramosus* | P | 37 ± 19 | 54 ± 12 | 84 ± 18 | 118 ± 26 | 172 ± 53 |
| *Bathygobius soporator* | A | 25 ± 25 | 36 ± 9 | 66 ± 11 | 110 ± 18 | 156 ± 10 |
| *Rypticus nigripinnis* | P | 54 ± 12 | 75 ± 8 | 101 ± 7 | 141 ± 8 | 191 ± 12 |
| *Rypticus subbifrenatus* | A | 35 ± 14 | 54 ± 10 | 83 ± 8 | 123 ± 11 | 187 ± 16 |

less extreme temperature on reproductive success and larval viability. Data should be gathered for species from different habitats and should include pelagic species which may be involved to a greater extent in canal transit.

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## Adenosine 3′,5′-Monophosphate–Dependent Protein Kinase of Cultured Mammalian Cells

Abstract. *Protein kinase was partially purified from Chang's liver cells, 3T6 mouse embryo fibroblasts, and HeLa cells. The rate of histone phosphorylation catalyzed by the kinase from each of these cell lines was stimulated two- to threefold by $1 \times 10^{-6}$ molar adenosine 3′,5′-monophosphate. The same concentration of guanosine 3′,5′-monophosphate failed to stimulate these kinases.*

Hormone-sensitive adenyl cyclase has been demonstrated to be present in three cultured mammalian cell lines—Chang's liver cells, 3T6 mouse embryo fibroblasts, and HeLa cells (*1*). Since adenosine 3′,5′-monophosphate (cyclic AMP)–dependent protein kinase has been partially purified from a wide variety of mammalian tissues (*2*), it would be of interest to ascertain whether a cyclic AMP–dependent protein kinase is also present in the above hormone-sensitive, cultured cell lines. The data obtained in our study demonstrate the presence of a cyclic AMP–dependent protein kinase in Chang's liver cells, in 3T6 mouse fibroblasts, and in HeLa cells.

Chang's liver and HeLa cells were grown in suspension culture, and 3T6 fibroblasts were grown in stationary culture as reported (*1*). Cyclic AMP–dependent protein kinase was obtained from sonicated cells and fractionated through the ammonium sulfate step as described in the purification procedure of Kuo and Greengard (*2*). Unfractionated calf thymus histone (Schwarz/Mann) was used as substrate, and enzymatic activity was determined according to the method of Kuo and Greengard (*3*). Values for phosphorylation of histone were corrected by subtracting the small amount of radioactivity present at zero incubation time. Protein was determined by the method of Lowry *et al.* (*4*).

Each of the cultured cell lines examined contained a protein kinase that was stimulated two- to threefold by $1 \times 10^{-6}M$ cyclic AMP (Table 1). The protein kinase from 3T6 fibroblasts had the highest control activity and was stimulated to a greater extent by cyclic AMP than that from the other cell lines. Neither the HeLa cell enzyme nor that of 3T6 fibroblasts was stimulated by $1 \times 10^{-6}M$ guanosine 3′,5′-monophosphate (cyclic GMP).

Table 1. Protein kinase activity of cultured mammalian cells. Enzyme activity is expressed as picomoles of $^{32}P$ transferred per 100 $\mu$g of enzyme protein during a 5-minute incubation period. The numbers in parentheses are the number of separate enzymatic assays. The cyclic nucleotides, when present, were at a concentration of $1 \times 10^{-6}M$.

| Cell line | Protein kinase activity | | |
|---|---|---|---|
| | Control | + Cyclic AMP | + Cyclic GMP |
| HeLa | 6.6 (4) | 12.4 (4) | 6.7 (3) |
| Chang's liver | 17.2 (3) | 40.0 (3) | |
| 3T6 fibroblast | 26.3 (3) | 84.7 (3) | 24.3 (2) |

Kuo and Greengard (*2*) have shown that protein kinase from bovine liver is stimulated tenfold by cyclic AMP, whereas that from rat liver is stimulated only 2.5-fold. Since protein kinase has not been purified from normal human liver, it is not possible at present to determine whether the enzyme from the cultured human liver (Chang's) cells (*5*) has similar properties to that of whole human liver. In contrast to the enzyme from normal liver, adenyl cyclase from Chang's liver cells is markedly stimulated by epinephrine but is not influenced by glucagon (*1*).

Both the extent of stimulation of adenyl cyclase by catecholamines and the total adenyl cyclase activity measured in the presence of sodium fluoride are greater in 3T6 fibroblasts than in Chang's liver cells, with still lower values obtained for HeLa cells (*1*). Thus a parallel appears to exist between hormone-sensitive as well as total adenyl cyclase activity and the extent of protein kinase stimulation by cyclic AMP in these cultured mammalian cell lines.

The cyclic AMP–dependent protein kinase exists in an inactive form that is split into regulatory and catalytic subunits as a consequence of the binding of cyclic AMP to a regulatory site (*6*). The catalytic subunit is then fully active and therefore insensitive to further stimulation by cyclic AMP. The ratio of the rate of phosphorylation in the absence of cyclic AMP to that in its presence reflects the fraction of protein kinase originally in the active form. The data from HeLa cells, Chang's liver cells, and 3T6 fibroblasts suggest that approximately 50 percent of the protein kinase prepared from each one of these cell lines is in the active form.

Ours is the first report of a cyclic AMP–dependent protein kinase from cultured cells other than neuroblastoma (*7*). As was found in these studies, the kinase from neuroblastoma is also stimulated two- to threefold by cyclic AMP and is insensitive to cyclic GMP. Although neuroblastoma cells have adenyl cyclase activity, the basal activity is low and insensitive to hormonal stimulation (*8*).

Chang's liver cells, HeLa cells, and 3T6 mouse embryo fibroblasts each