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GENERAL BIOLOGY

A Long-Lived Parasite Extending the Host Life Span: The Pearl Mussel *Margaritifera margaritifera* Elongates Host Life by Turns out the Program of Accelerated Senescence in Salmon *Salmo salar*

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The Pacific salmon from the genus *Oncorhynchus* (family Salmonidae), which inevitably dies after the first spawning is a good example of the natural phenomenon referred to as phenoptosis, i.e., the programmed death of an individual, which is the way to remove excess individuals that the community does not need anymore by triggering a self-destruction program [10]. All species of the genus *Oncorhynchus* (chum, pink, coho, chinook, sockeye, masu salmons, etc.) die in large amounts because of accelerated senescence (progeria) soon after the completion of a long-term anadromous migration from the ocean into the river in order to spawn. Their death is not related to an energy loss, because even after chum and pink salmons spawn in the shortest streams (springs) with a hardly noticeable flow, the fish all the same die two weeks or a month after spawning. Fish dying is a result of triggering a special biochemical program the key process of which is the production of steroid hormones, such as a stress hormone cortisol. Accelerated senescence of Pacific salmon can be prevented by removal of the gonads or the adrenal glands in immature fish. This procedure increases the chinook salmon life span by a factor of two (from four to eight years) [8, 9]. The biological implication of the salmon parental suicide lies in the fact that fish bodies serve as food source for river invertebrates, which, in turn, are eaten by juvenile salmon [13]. Important signals triggering the progeria program are the migration from sea to fresh water, the release of sexual products, and stress caused by confrontations in breeding areas.

However, under natural conditions, a surprising example is known of switching off the biochemical program of postreproductive suicide by a symbiotic organism, larvae of a freshwater pearl mussel *Margaritifera margaritifera*, which is a tissue parasite of gill epithelium of the Atlantic salmon *Salmo salar*. Because of this phenomenon, the life span of the pearl mussel host salmon is extended up to 13 years, and the fish is capable of repeated spawning, from two to six times.

The goal of this study was to illustrate, by the example of a mollusk–fish system, that the parasite can both inhibit host senescence and stimulate nonspecific stress resistance of the host, i.e., it regulates the host life span.

Our long-term observations were made when we developed a biotechnology to restore the endangered pearl mussel species. The corresponding data are shown in Tables 1 and 2.

The fresh-water pearl mussel (family Margaritiferidae, class Unionoida) is the most long-lived species among invertebrate animals. It reaches a maximum age of 200 years [3, 14]. Paleontological evidence suggests that, in Europe, salmon of the genus *Salmo* (the Atlantic salmon and brown trout) coevolved with the pearl mussel for 8×10^6 years, since Pliocene; the present range of the mussel coincides with the ranges of these fish species [12]. By the 21st century, several dozens of reproducing *M. margaritifera* populations remained in Russia, Fennoscandia, and Scotland. When the pearl mussel larva (glochidium) growth was studied on the gills of Atlantic salmon parr and adult fish in various water bodies of northwestern Russia, we found that the larva-infested spawners did not die in autumn after spawning and did not migrate downstream to the sea; they display no signs of progeria and live in the river until the next summer [12]. Note that, in winter, wild salmon carry about 2×10^3 to 7×10^3 small pearl mussel glochidia (50–70 μm in diameter) per fish. By summer, these fish remained nimble, although they lost much weight, and showed normal aggressive behavior when attacking the spoon lure of an angler. In the White Sea basin, the summer ecological form lived in rivers for a year (from June to the next June), whereas the autumn form, almost two years (for example, from August 2002 to June 2004). Thus, the autumn salmon form can carry

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Table 1. Survival of two groups of *S. salar* spawners (experimentally infected or uninfected with pearl mussel larvae) in cages after three types of stress

Compared fish groups	Asphyxia	Thermal burn of gill	Wounds caused by hooks
	Survived on the fifth day, %	Survived on the fifth day, %	Survived on the 25th day, %
Uninfected	0 out of 60 (0)*	0 out of 60 (0)*	60 out of 72 (83)*
Infected	32 out of 60 (53)*	8 out of 60 (13)*	70 out of 72 (97)*

Note: Asterisks indicate significant differences between the compared groups (infected versus uninfected), $P < 0.05$.

Table 2. Fungal disease (*Saprolegnia*) and skin tumor (epithelioma) in two groups of wild *S. salar* parrs: those infested by pearl mussel larvae (the main bed of the Varzuga River) and uninfected ones

Years	Habitat	Average population density of		Number of examined fish	Total number of examined fish/number of larva-infested fish	Number of mollusk larvae per fish		Number of fish affected with *Saprolegnia* and tumors (in parentheses, expressed in percent of the number of fish caught)
		adult mollusks; number per 100 m^2	fry, number per 100 m^2			average	minimum–maximum	
1997	The main bed	854	114	1050	250/250	1267	430–4020	0 (0)
1997	Tributaries	0	125	975	50/0	0	0	20 (2)*
1999	The main bed	790	167	1108	100/96	716	470–2160	0 (0)
1999	Tributaries	0	117	630	50/0	0	0	6 (1)*
2001	The main bed	820	151	570	200/200	860	65–2850	0 (0)
2001	Tributaries	0	136	450	60/0	0	0	14 (3)*
2003	The main bed	598	98	548	100/94	168	92–1850	0 (0)
2003	Tributaries	0	95	354	50/0	0	0	17 (5)*

Note: Asterisks indicate significant differences between the compared parr groups (inhabitants of the main bed versus inhabitants of marshy tributaries). $P < 0.05$.

pearl mussel larvae twice during each visit from the sea to the river. In summer, both forms of spawners migrate downstream to the sea and only after this migration do most fish die in the sea from exhaustion. The strongest fish survive. The fish that reproduce two or three times constitute 10–40% of the population. Cases of spawning repeated five and even six time have been reported [4].

The biological implication of senescence inhibition in fish infested by pearl mussel larvae is the following. While growing on fish gills, the larvae increase about ten times in size. They need about 1500 degree–days to complete the parasitic phase. In cold rivers of northern Europe, the annual sum of degree–days is 1750; therefore, the mollusk larval phase proved to be extremely long (300–350 days). Unlike the parasitic helminth tapeworm ligula, the helminth that kills the intermediate host (fish) to enter the definitive host (bird), the parasitic mollusk must extend the host life, which must not be shorter than the mollusk larval phase, i.e., 8–11 months. Pearl mussel "takes care" that the host salmons (both adult spawners and parrs) that carry larvae on their gills since autumn might not undergo rapid senescence and remain healthy as long as possible—at least until the next summer, to ensure the completion of the mollusk metamorphosis on the fish gills. Later, mature mollusks leave the hosts to become free-living organisms inhabiting the river bottom. During millions of years of coevolution, the mollusk symbiont served as a directional selection factor for host adaptability and longevity, and some longevity assurance genes of the mollusk, e.g., the genes that control resistance to starvation or asphyxia, might have been integrated into the host genome [15].

Note that in the pearl mussel itself, even in old individuals, no diseases, parasites, or tumors were observed until recent climate warming. The individuals of a venerable age die most likely from permanent allometric growth that leads to an excessive increase in shell weight, rather than from aging-related diseases [2].

Now, let us consider various explanations of the above data alternative to the hypothesis on senescence turnoff. For example, it could be postulated that *Salmo* from Fennoscandialive longer than *Oncorhynchus* salmon from Sakhalin because the ambient temperature in the north is lower and metabolism is decelerated. This explanation conflicts with the data on the Sakhalin salmon acclimatization in the White Sea in the 1970s,

where pink salmon died after spawning in the same pearl rivers (the Varzuga, Umba, Keret', etc.) [3, 13], i.e., low temperatures failed to extend the pink salmon life span. Hence, it is impossible to modify the trait post-spawning mortality merely by a temperature decrease.

Another objection is that, in fact, the European pearl mussel does not extend the host life; it merely uses the longer fluvial stage of the Atlantic salmon; the larvae unhurriedly grow on the host while it remains alive. However, this interpretation fails to explain the Atlantic salmon behavior: why does a salmon that has lost 50% of body weight (it does not feed in fresh water) purposelessly wanders in the river for another eight or nine months? It would undoubtedly be better for the fish to migrate downstream to the sea where it can feed in Gulf Stream areas rich in food (because there is another spawning in store for it). Nature is unlikely to be so wasteful.

Salmon life extension is easily understood if we assume that the fish is a surrogate mother for mollusk embryos, for which it ensures food, growth, safety, and dispersal. In turn, the mollusk larva seems to secrete into the fish body the substances that inhibit fish senescence and stimulate stress resistance. Note that adult mollusks provide parrs with shelter on the bottom of the river; they account for fouling of the bottom (i.e., food for fish) and improve water quality by filtration [13].

Experiments in fish cages showed that the parasite was not only harmless (e.g., it caused no changes in blood count or spermatozoon motility [12, 13]), but also directly improved the host health by increasing the resistance of the host to unfavorable environmental factors. For example, after spawning, mussel-infested riverine salmon exhibited a relatively high survival rate (53% versus a 100% mortality of intact salmon) despite strong stresses, such as asphyxia (45–60 s outside of water) or thermal burn of gill because of the contact with human fingers (13%). The viability of hook-injured mollusk-infested salmons was also increased by 14% (Table 1). Long-term field observations showed that pearl mussel improved nonspecific resistance to dangerous diseases, such as epithelial tumors and fungal (*Saprolegnia)* infection in both adult fish and parrs (Table 2). For example, in the Varzuga River, where there are still several tens of millions of pearl mussels and about 10^7 salmon parrs, the population density of the latter is unprecedented in the world (100–170 fish per 100 m^2, whereas the normal population density is 20–40 fish). When living in such overpopulated areas, salmons, which are characterized by territorial defense behavior, might be expected to undergo chronic stress because of territorial confrontations. However, in fact, salmon parrs coexist well (without a loss of normal aggression) in spawning and rearing areas without any signs of exhaustion of their nervous system.

The fact that pearl mussel larvae optimize the neuroendocrine control of salmon behavior is confirmed by the absence of parr morbidity in the main Varzuga River bed, where 90% of parrs live. Here, almost all fry inhabiting clear water among pearl mussel colonies were carriers of glochidia. Among 3200 parrs that we examined visually in 1997–2003, none had skin ulceration, ectoparasites, skin tumors, or fungal diseases. At the same time, 10% of parrs lived in marshy tributaries. Food availability is there higher than in the main river, and parrs are larger in size. However, the water is turbid, has a brown or "tea" color, and contains no pearl mussel colonies. In tributaries, the epidemiological situation was worse: about 50 parrs out of 2400 examined ones had skin tumors and were infected by *Saprolegnia* (Table 2).

Pearl mussel larvae decelerate growth, maturation, and extend the riverine life of salmon fry. This can be illustrated by an example of the largest European population of Atlantic salmon from the Varzuga River. Because of specific stream conditions (shallow water, the lack of lakes, plenty of bogs in the drainage area, minute spawning ground, etc.) the population mostly consists of small fish weighting 2–5 kg. In addition, selective fishing with seines in the 20th century has "beaten out" large fish of older age groups from the population. Nevertheless, the age structure of the Varzuga River salmon population is still unexpectedly complex (12 age groups). The following ages of adult fish are observed (the number of years in the river + the number of years in sea): 2 + 1+, 2 + 2+, 2 + 3+, 3 + 1+, 3 + 2+, 3 + 3+, 4 + 1+, 4 + 2+, 4 + 3+, 5 + 1+, 5 + 2+, 5 + 3+. Ichthyologists have long since noted that parrs migrate downstream from the Varzuga River to the sea when their body is much smaller (10 cm) than that of fish in the neighboring rivers (12–14 cm). Surprisingly, that these small fish were not eliminated, although they were unready to inhabit the sea pelagic zone to which they rapidly migrated from the river [3].

The results of our long-term studies on the age structure of smolts that migrate downstream in summer suggest that smolts from marshy tributaries migrate downstream to the sea as soon as at an age of two or three years, whereas the parrs from the main stream live on the mollusk colonies for three to five years until they leave the river. In general, the average parr life in the river before migrating into the sea is much longer than that of parrs in tributaries (3.3 and 2.5 years, respectively) because of pearl mussel influence. A young mollusk needs a minimum period of 0.8 year (300 days) to complete the parasitic phase in fish. On some large pearl mussel colonies, the proportion of parrs at an age of four or five years is as high as 70%, and their average life in the river can reach a maximum of 3.8 years. Immunological parasite–host relationships in a the pearl mussel–fish system were studied by our European colleagues [7, 11] and by us [12, 13]. The host proved to determine the safe proportion of parasites and maintain them alive. Feeble glochidia that show slow cyst formation are killed with the fish immune system, whereas successful glochidia resist the fish immune

response [12], which is confirmed by the facts on repeated parr invasion with pearl mussel larvae in Norway [11] and Scotland [7], as well as the adult salmon infection in Russian pearl rivers [13]. The parasitic larva, in turn, not only stimulates host gill cell migration and proliferation during incapsulation. Upon larval metamorphosis, the mollusk induces mass necroses of host cells, because the cyst walls are disrupted when it leaves the multilayer capsule. However, within one or two days after the parasite leaves the fish, thousands of empty cysts with ragged edges rapidly dissolve [12], which is presumably a result of the stimulation of apoptosis and regeneration of the injured gill tissue. We never observed secondary infection of gills after glochidia leaved fish under natural conditions or in experiments at fish factories.

In salmonids, various expressions of the reproductive function, such as imprinting, homing, guarding the territory and eggs, and interaction between individuals are known to be under the control of the nervous and endocrine systems [5]. In these fish, the same neuroendocrine cell of the hypothalamic–pituitary system (the preoptic nucleus of the hypothalamus) is capable of producing simultaneously such substances as endorphins, enkephalins, neurotensins, substance P, and analogs of tropic hormones of adenohypophysis [6]. The salmon hypothalamus is overexcited after spawning, and symbiosis with glochidia seems to inhibit its activity to support the optimal level of pituitary hormones. This might inhibit the triggering of the senescence program in fish.

Note that Pacific salmon dies from an increased activity of the chain hypothalamus–pituitary gland–adrenal cortex–enhanced cortisol production–thymus atrophy (with a decrease in immunity)–increased sugar, fatty acid, cholesterol, and insulin contents of the blood–death from myocardial and renal infarctions and stroke.

Dil'man was one of the first to pay attention to the fact that hormonal changes during salmon and human senescence are almost the same [1], the only difference being that senescence of humans is much slower than the acute process in salmon. As shown recently for the sockeye salmon from Colorado (United States), formation of amyloid plaques on brain neurons of a salmon that dies after spawning strikingly resembles the progress of Alzheimer's disease in humans [8]. In all vertebrates, from salmon to human, the neuroendocrine and immune systems are similar in structure. Undoubtedly, pearl mussel larvae somehow neutralize senile changes in the following salmon regulatory system: hypothalamus–pituitary gland–peripheral endocrine glands–hypothalamus. We have inherited from our fish ancestors the principles of regulation of subcortical structures. If pearl mussel larvae have learned how to switch off the salmon senescence program, why cannot we try and affect human senescence in order to prolong human life? An idea was recently put forward that we have inherited senescence as a harmful atavism from our ancestors, and overcoming the atavistic mechanisms would eliminate senescence. The search for specific inhibitors of the programmed death of an individual is an approach to solving this problem [10]. Understanding how evolution has solved it in the case of fish–mollusk symbiosis would help to attain this goal.

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