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ZOOLOGY

A Paradox of Parasite Prolonging the Life of Its Host. Pearl Mussel Can Disable the Accelerated Senescence Program in Salmon

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Abstract—A unique case is analyzed when the accelerated senescence program (progeria) in salmon (Salmonidae) can be canceled by larval parasite of the gills—freshwater pearl mussel *Margaritifera margaritifera.* As a result, the maximum age of *Salmo* fishes hosting the mussel can be as high as 13 years. The mollusk–fish system made it possible to demonstrate that the parasite can inhibit senescence of the host and stimulate nonspecific resistance to stress, i.e., can control longevity. The mussel proved to increase the resistance to epitheliomata and cutaneous mycoses. The parasite is perceived to neutralize the senile changes in the salmon regulatory cascade hypothalamus–pituitary–peripheral endocrine glands–hypothalamus.

INTRODUCTION

Pacific salmon (Salmonidae) inevitably dies after the first spawning as a result of triggering the genetic program of death (Robertson *et al.*, 1961; Maldonado *et al.*, 2002).

Salmons of the genus *Oncorhynchus* inhabiting the Pacific from the Pleistocene (3 million years to the present)—chum, pink, coho, king, sock-eyed, cherry, etc. salmons—each year die in masses as a result of accelerated senescence (progeria) soon after a long-term anadromous migration for spawning. Their death is not due to excess energy expenditures since chum and pink salmons spawning in very short rivers or springs with hardly noticeable current still die within two–four weeks after spawning. Their death results from triggering a special biochemical program, in which the production of steroid hormones, particularly, stress hormone cortisol, plays the key role. Accelerated senescence of Pacific salmon can be prevented by gonadectomy or adrenalectomy. Such operation doubles the lifespan of king salmon from 4 to 8 years (Robertson *et al.*, 1961; Maldonado *et al.*, 2002). The biological significance of parental suicide is to provide food for river invertebrates, which are consequently consumed by salmon fry (Ziuganov *et al.*, 1998). Important signals for triggering the program of accelerated senescence include salmon transition from seawater to freshwater, spawning, as well as competition and confrontation stress on the spawning ground.

Here we postulate that there is an amazing example of disabling the biochemical program of accelerated senescence and post-reproductive suicide in another salmon species, Atlantic salmon *Salmo salar*, under the influence of a symbiotic organism—periodic larval parasite of salmon gills, freshwater pearl mussel *Margaritifera margaritifera.* The maximum lifespan of salmons harboring the symbiotic mussel can be extended to 13 years and they can spawn many (up to 5–6) times.

In this work we present evidence for inhibition of senescence and stimulation of nonspecific resistance to stress, i.e., for longevity control, in a vertebrate host (fish) by an invertebrate parasite (mollusk). We analyzed and reviewed our own (Tables 1–3) and published data in order to understand the mechanisms of this phenomenon.

Freshwater pearl mussel is the most long-lived invertebrate with the top lifespan of 200 years (Ziuganov *et al.*, 2000; Ziuganov, 2004). Paleontological data indicate that the mussel and *Salmo* salmons (Atlantic salmon and sea trout) coevolved in Europe for at least 8 million years from the Pliocene so that the mollusk's range fits in the ranges of these fish species (Ziuganov *et al.*, 1994).

MATERIALS AND METHODS

Atlantic salmon spawners were caught in 1987–2003 in the basins of the Varzuga, Umba, and adjacent rivers from June to November. Fish were caught to study the behavior and survival of salmon as a temporary carrier of pearl mussel larvae (glochidia). Experimental goals included establishing the effect of catching and holding in a tank on salmon survival in order to determine the conditions for semiartificial reproduction of the rare and endangered pearl mussel species using its natural host, salmon, without adverse effect to the fish. All fish were held in cylindrical tanks for 5 to 60 days in running river water, in 2 × 5 m river plots

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Table 1. Survival rates of two groups of *Salmo salar* spawners (experimentally infested with pearl mussel larvae and intact) in tanks after exposure to three stress factors: (1) asphyxia in the air for 45–60 s, (2) thermal burn of the gills by hot hands of a fisherman, and (3) hook wounds of the snout, eyes, mouth, and gullet

Fish group	Asphyxia, survival rate on day 5, %	Gill burn, survival rate on day 5, %	Hook wounds, survival rate on day 25, %
Intact	0 from 60 (0)*	0 from 60 (0)*	60 from 72 (83)*
Infested	32 from 60 (53)*	8 from 60 (13)*	70 from 72 (97)*

*Differences between the intact and infested groups were significant at $p < 0.05$. After the acute experiment, fish were held for 5–25 days in tanks. Spent salmons were infested at 1.5–2.0 thousand pearl mussel larvae. Salmons recently arrived from the sea were uninfested. Survived fish were used in other experiments. Combined data obtained in 1997–2003.

Table 2. Comparison of two groups of wild *Salmo salar* parr infested with pearl mussel larvae (from the main bed of the Varzuga River over mussel colonies) and uninfested (from the bayous without the mussels: Pyatka, Krivets, Yapoma, Aren'ga, and Mel'ga Rivers) caught by electrical fishing, 1997–2003

Year	Habitat	Mean density		Number of examined fish	Total number of dissected fish/number of fish infested with mussel larvae	Number of mussel larvae per fish		Number of fish affected by *Saprolegnia* and epitheliomata and number of caught fish (in parentheses)
		adult mussels, ind./100 m^2	fish fry, ind./100 m^2			mean	minimum–maximum	
1997	Main bed	854	114	1050	250/250	1267	430–4020	0 (0)
1997	Bayous	0	125	975	50/0	0	0	20 (2)*
1999	Main bed	790	167	1108	100/96	716	470–2160	0 (0)
1999	Bayous	0	117	630	50/0	0	0	6 (1)*
2001	Main bed	820	151	570	200/200	860	65–2850	0 (0)
2001	Bayous	0	136	450	60/0	0	0	14 (3)*
2003	Main bed	598	98	548	100/94	168	92–1850	0 (0)
2003	Bayous	0	95	354	50/0	0	0	17 (5)*

*Differences between groups (salmon parr from the main bed and bayous) were significant at $p < 0.05$.

bordered with webbing of 10 mm mesh size, or in the standard wooden tanks of the Umba Fish Hatchery. In total, over 400 spawners were used in experiments. Experimental details were described elsewhere (Ziuganov *et al.*, 1994, 1998, 2000, 2001a, 2001b).

In 1997–2003, the density of parr diffusion of Atlantic salmon *Salmo salar* was studied in the Varzuga River and bayous. Each plot was fished three times using the method of Zippin (Ziuganov *et al.*, 2001b) and the density of diffused fry (including 0+ yearlings) was calculated for each area. Fifteen reference plots were fished in the river basin. The caught fry were kept in tanks and, after measuring total body length, were immediately let back to water. Age was determined from scales in 760 individuals. The area of fished plots varied from 120 to 475 m^2. The annual total fished area of the spawning and rearing grounds was 3000 m^2 (Ziuganov *et al.*, 2001b). The obtained data were statistically analyzed using Student's *t*-test.

Table 3. Age structure of two smolt groups described in Table 2, 1997–2003

Fish groups	Proportion of different age groups, %				Total fish number
	2 years	3 years	4 years	5 years	
Infested (from the main bed)	9*	62*	24*	5*	420
Uninfested (from the bayous)	54	46	–	–	340

*Differences significant at $p < 0.05$.

RESULTS AND DISCUSSION

Observations in the North European rivers (1987–2004). By the 21st century, several tens of large reproducing populations of *M. margaritifera* remained only in Russia, Fennoscandia, and Scotland. While studying

the development of pearl mussel larvae (glochidia) on the gills of young and adult Atlantic salmon, we noticed that the infested spawners did not die after spawning in autumn and did not migrate down to the sea but remained in the river without signs of progeria (accelerated senescence) until next summer (Ziuganov *et al.*, 1994). In this case, wild salmons harbored on their gills up to 2–7 thousands of small (50–70 μm) pearl mussel glochidia per fish in winter. By next summer, these thin but active fish demonstrated the normal aggressive response to attack a spinner. In the White Sea basin, the summer ecological form of the salmon spends one year in rivers (from June to June), while the autumn form spends almost two years there (e.g., from August 2002 to June 2004). Hence, the autumn form of the salmon can harbor pearl mussel larvae twice per visit from the sea to the river. In summer, both forms of salmon spawners migrate down to the sea and most fish die of starvation there. The strongest fish can survive. In general, the proportion of fish spawning for the second and third time is 10–40%, although fifth and sixth spawnings were reported (Fleming, 1998).

The biological significance of disabling the senescence program in fish infested with pearl mussel larvae is as follows. The larvae grow on the fish gills and their size increases tenfold. They need about 1500 degree-days to complete the parasitic phase. In cold rivers of northern Europe, the total annual degree-days equals 1750, which makes the larval phase of the mollusk very long, 300–350 days. In contrast to tapeworm *Ligula*, which kills the intermediate host, fish, to enter the definitive host, bird, the parasitic mussel is critically interested in extension of the host lifespan at least until the larval stage of the mussel is completed, i.e., for 8–11 months. The pearl mussel ensures that the host salmon (spawners and parr) that harbored some larvae in autumn does not die of senescence as long as possible—at least until next summer so that the mussel can complete its long metamorphosis in the fish gills, leave the host, and start the free-living stage on the river bottom. During their coevolution for millions of years, the symbiotic mollusk was a factor driving selection for the adaptation and longevity of the host. Moreover, it could well integrate some of its "longevity genes" into the host genome, such as the genes controlling resistance to starvation or asphyxia (Ziuganov *et al.*, 2001a).

Note that, until recent climatic warming, no diseases, parasites, or epitheliomata have been reported in pearl mussels even at the age of 100 years. Most likely, old individuals die from the consequences of genetically programmed continuous growth such as disproportionately heavy shell rather than from senile diseases (Ziuganov, 2004). By the beginning of the 21st century, 99% populations of pearl mussel became extinct due to their high sensitivity to water contamination; at the same time, in many rivers where pearl mussel does not live any longer, salmon retained an atavism of starvation wintering after spawning.

Situation in the Pacific region. A series of interesting observations and experiments were made in the Pacific region. Shorter-lived pearl mussels live here as compared to Europe. *M. laevis* lives in Asia (Sakhalin and Japan) and its maximum lifespan is 30–40 years. *M. falcata* lives in California to reach the age of 45–60 years. Both species are parasitic on salmons of the genus *Oncorhynchus*. However, due to higher temperatures of river water (5000 degree-days), the developmental time of the larvae on the fish gills is 8–10 times shorter (no more than 30–45 days). In Japan, chum and sock-eyed salmons, which spawn in September, are of the host of the local freshwater pearl mussel. By this period, pearl mussel already completes the metamorphosis and leaves the host before fish spawning and death. In California, mussel's host, king salmon, spawns in July and the mussel completes the parasitic stage in May–June (Ziuganov *et al.*, 1994). Thus, the larvae have no need to extend the host's lifespan to complete their own metamorphosis. In both cases, larval development is fast enough to let them leave the fish before the peak of the cellular and humoral immune response of the host (7–12 weeks). That is why, no fine biochemical interactions in the parasite–host system similar to those in Europe should be expected here. Indeed, the Pacific pearl mussels do not affect the biology of host salmon, in contrast to the European one which extends the fish lifespan by several years. It is of interest that the European *M. margaritifera* could not develop on the gills of chum, sock-eyed, and king salmons, while Californian *M. falcata* died on the gills of Atlantic salmon under experimental conditions. In other words, European and Pacific mussels are specific to local host species (Ziuganov *et al.*, 1994).

Alternative hypotheses. Let us now consider possible explanations of these data other than the hypothesis of disabled senescence. For instance, *Salmo* salmons could have a longer lifespan in Fennoscandia than *Oncorhynchus* salmons in Sakhalin since the environmental temperature is lower in the north and their metabolism is decelerated. However, this is not consistent with the results of Sakhalin pink salmon acclimation to the White Sea in 1970s (Varzuga, Umba, Keret', and other rivers) when all fish died after spawning (Kalyuzhin, 2003); i.e., low temperatures did not extend the lifespan of pink salmon. Hence, the "post-spawning death" character cannot be modified by temperature decrease.

Alternatively, the European mussel could benefit from a longer river phase of the Atlantic salmon life cycle and simply lives on the host as long as it is alive, rather than extends its lifespan. However, such interpretation does not explain fish behavior after spawning: why fish that lost 50% of its weight aimlessly swim in the river for 8–9 months (considering that it does not feed in freshwater)? It should be much practicable to migrate down to the sea and fatten in the warm feeding waters of the Gulfstream, especially considering the

awaiting spawning. Nature can hardly have afford such useless waste.

Extension of the salmon lifespan becomes biologically sensible assuming that the fish is a "surrogate mother" for the mussel embryos, which provides for their feeding, growth, protection, and diffusion. Consequently, the mollusk larvae seem to provide the "mother" body with substances inhibiting senescence and promoting stress resistance. Moreover, adult mollusks provide salmon fry with additional shelters on the bottom as well as with overgrowth (food) and improve water quality through biofiltration (Ziuganov *et al.*, 1998).

Evidence for mutual benefit of symbiosis. Experiments demonstrated that the "symbiont-parasite" not only is not harmful (e.g., for the hemogram or locomotor activity of spermatozoa; Ziuganov *et al.*, 1994, 1998) but directly renders the host healthy and increases its resistance to unfavorable environmental factors. For instance, the survival rate of spent salmon infested with the pearl mussel after exposure to severe stress such as asphyxia or thermal burns of the gills was 53 and 13%, respectively (as compared to the 100% death rate of the intact salmon). The survival rate of salmons with hook wounds also proved by 14% higher in mollusk carriers (Table 1).

Long-term field studies demonstrated that the pearl mussel increased nonspecific resistance to dangerous diseases such as epitheliomata or fungal lesions induced by *Saprolegnia* both in salmon adults and fry (Table 2). For instance, salmon fry demonstrates unprecedently high population density of 100–170 individuals per 100 m^2 (vs. the usual density of 20–40 ind./100 m^2) in the Varzuga River, where tens of millions of pearl mussels and around ten millions of fry still live. In such overpopulated habitats, salmon parr with territorial defense behavior should be permanently stressed by territorial confrontations. At the same time, salmon parr get along well with each other (without losing the normal aggressiveness) on the spawning and feeding grounds without signs of nervous exhaustion. Apparently, pearl mussel larvae supply host neurons with natural antidepressants via blood.

The optimization of neuroendocrine control of salmon behavior by mussel larvae is confirmed by the absence of diseases of salmon parr in the main bed of the Varzuga River harboring 90% parr. Here they live in clear water among pearl mussel colonies and nearly all parr carry larvae of the symbiont. Not a single parr with surface ulcerations, ectoparasites, epitheliomata, or fungal diseases was revealed among 3200 parr visually inspected in 1997–2003 (Table 2). At the same time, around 10% parr (around one million individuals) live in the bayous. Due to favorable feeding conditions, fry are larger there as compared to the main river bed. However, brown water is not so clear and mussel larvae are missing here. The epidemiologic conditions are not perfect here: over 50 out of 2400 inspected ones proved affected by epitheliomata and *Saprolegnia*, suggesting that many parr are stressed in conditions of overpopulation (Table 2) (Ziuganov and Kalyuzhin, 2004).

Increased duration of the parr stage. Pearl mussel larvae decelerate growth and maturation and extend the river stage of young salmons. Let us consider a specific example of the largest European population of Atlantic salmon in the Varzuga River. Specific hydrologic conditions of the river (shallow water, the absence of lakes, abundance of bogs in the basin, fine spawning substrate, etc.) provided for formation of schools largely including small fish weighing 2–5 kg. In addition, targeted netting in the 20th century eliminated large fish of older groups. Nevertheless, this school still maintains unexpectedly complex age structure including 12 age categories (years in the river + years in the 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+. Long ago ichthyologists noticed that salmon smolts migrating from the Varzuga River down to the sea had considerably shorter body length (10 cm) as compared to neighboring rivers (12–14 cm). They were surprised why such small fish seemingly unfit for the pelagic life were not eliminated after their abrupt transition from the river to the sea (Kalyuzhin, 2003). Our long-term observations of the age structure of salmon smolts migrating downstream in summer are summarized in Table 3. Smolts from the bayous migrate down to the sea already at the age of 2–3 years, while parr from the main bed living over mussel colonies leave river at the age of 3–5 years. In general, pearl mussel significantly increased the duration of salmon parr life in the river as compared to bayous (3.3 and 2.5 years, respectively). The period of 0.8 years suffices for young mussels to complete the parasitic stage in the fish. The proportion of smolts at the age of 4–5 years was 70% over certain large pearl mussel colonies and the mean duration of the river stage also reached the maximum of 3.8 years. Table 3 compares the age structure only of parr since they do not migrate in the basin before their travel to the sea, while adult salmons easily migrate between the main bed and the bayous.

Immune properties of the parasite–host interactions. In nature, pearl mussel larva is passively carried to the gills with water flow and binges a part of the gill plate with its shells. Previously we demonstrated that attachment of pearl mussel larva to an appropriate host induces migration of gill epithelial cells to the wound and a one-layer capsule is rapidly formed (within 5–12 h) (Ziuganov *et al.*, 1994). Then the larvae stimulates formation of a multilayer capsule resulting from the proliferation of gill epithelial cells. In the case of inappropriate fish host, the capsule is either not formed or, even if it started to form, a pronounced hyperplasia of the host tissues surrounding the glochidium (foreign body reaction) is observed 1–5 days after the invasion and the larva is cast off. Pearl mussel is a gill parasite and it tightly contacts this immunoreactive organ. We believe that the rate of epithelial capsule formation

from host cells is the critical point. If it occurs before the peak immune response, the glochidium undergoes metamorphosis. Death of "weak" glochidia at the initial stages is due to nonspecific macrophage-dependent reactions. As a result, only a fraction of pearl mussel larvae survive the immune response of the fish (Ziuganov *et al.*, 1994). This is confirmed by successful repeated invasion of salmon fry with pearl mussel larvae in Norway (Wachtler *et al.*, 2001) and Scotland (Hastie and Young, 2001) as well as by successful invasion of adult salmons in Russian rivers where pearl mussel lives (Ziuganov *et al.*, 1998). The parasitic larva not only stimulates host cell migration and proliferation. It also causes mass necroses of host cells twice in the fish: during invasion, when it wounds the gill epithelium, and during the release from the capsule, when it breaks the capsule walls. Within one or two days after parasite release from salmon, thousands of empty cysts with ragged edges are simultaneously resolved, which can be attributed to the stimulation of apoptosis in damaged gill tissues. Reproduction-related functions such as imprinting, homing, territory and spawn defense, and individual interactions are controlled by the nervous and endocrine systems in salmonids (Khristoforov and Murza, 1998). Single neuroendocrine cell in the preoptic nucleus of the hypothalamic–hypophyseal system can simultaneously produce endorphins, enkephalins, neurotensins, substance P, and analogs of adenohypophyseal trophic hormones (Ekengren and Terlou, 1978). Apparently, the symbiosis with glochidia can decrease the activity of the overexcited hypothalamus in salmon after spawning in order to maintain the normal level of hypophyseal hormone production. This could inhibit triggering the senescence program in the fish. Let us recall that Pacific salmon dies after activation of the cascade hypothalamus–pituitary–adrenal cortex–high cortisol levels–thymus atrophy (accompanied by depressed immunity)–high blood sugar, fatty acids, cholesterol, and insulin–death of cardiac and renal infarction and insults.

How to disable the senescence program in humans? Dil'man (1986) was among the first to notice nearly identical hormonal changes during senescence of salmon and human. The only difference is a much slower rate of changes in humans than in pink salmon. Recently Maldonado *et al.* (2002) demonstrated a striking similarity between the deposition of β-amyloid plaques in the brain neurons of salmon dying after spawning and the course of Alzheimer's disease in humans. Senescence and related systemic diseases are thought to result from overstrained (!) rather than fading activity of the systems controlling energy processes, adaptation, and reproduction. The increasing activity decreases the responsiveness of the hypothalamus and other subcortical structures to the negative feedback signals. All these events are programmed (Dil'man, 1986).

The neuroendocrine and immune systems have the same pattern in all vertebrates from salmons to humans. It is clear that pearl mussel larva can somehow neutralize the senile changes in the salmon control cascade hypothalamus–pituitary–perinephric endocrine glands–hypothalamus. We believe that mussel larvae supply host neurons with natural antidepressants and, possibly, neurotransmitters or their precursors via blood. Preliminary analysis demonstrated that the parasite–symbiont releases water-soluble substances (including amino acids, peptides, and glycoproteins) to the host blood. Studies of the antigen pattern of the pearl mussel using sandwich immunoassay revealed nine water-soluble antigens of glochidia, five of which are glochidium-specific and are undetectable in adult pearl mussels.

We inherited the mechanisms of control of the subcortical structures from our fish ancestors. If pearl mussel larvae managed to disable the senescence program in salmon, humans can try to apply similar mechanisms to significantly increase our longevity.

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REFERENCES

Dil'man, V.M., *Bol'shie biologicheskie chasy* (Big Biological Clock), Moscow: Znanie, 1986.

Ekengren, B. and Terlou, M., Hypothalamic Centers and Innervation of the Hypophysis in the Atlantic Salmon and Rainbow Trout, *Ann. Biol. Anim. Biochem. Biophys.*, 1978, vol. 18, no. 4, pp. 837–842.

Fleming, Ya., Reproduction of the Atlantic Salmon, in *Atlanticheskii losos'* (Atlantic Salmon), Kazakov, R.V., Ed., St. Petersburg: Nauka, 1998, pp. 127–141.

Hastie, L. and Young, M., Freshwater Pearl Mussel *Margaritifera margaritifera glochidiosis* in Wild and Farmed Salmonid Stocks in Scotland, *Hydrobiologia*, 2001, vol. 445, pp. 109–119.

Kalyuzhin, S.M., *Atlanticheskii losos' Belogo morya: problemy vosproizvodstva i ekspluatatsii* (Atlantic Salmon of the White Sea. The Problems of Reproduction and Exploitation), Petrozavodsk: Petropress, 2003.

Khristoforov, O.L. and Murza, I.G., Reproductive Function in Its Regulation in Atlantic Salmon, in *Atlanticheskii losos'* (Atlantic Salmon), Kazakov, R.V., Ed., St. Petersburg: Nauka, 1998, pp. 75–127.

Maldonado, T.A., Jones, R.E., and Norris, D.O., Timing of Neurodegeneration and Beta-Amyloid (Ab) Peptide Deposition in the Brain of Aging Kokanee Salmon, *J. Neurobiol.*, 2002, vol. 53, pp. 21–35.

Robertson, O., Wexler, B., and Miller, B., Degenerative Changes in the Cardiovascular System of the Spawning Pacific Salmon (*Oncorhynchus tshawytcha*), *Circ. Res.*, 1961, vol. 9, pp. 826–834.

Wachtler, K., Dreher-Mansur, M., and Richter, T., Larval Types and Early Postlarval Biology in Naiads (Unionoidea), *Ecol. Stud.*, 2001, vol. 145, pp. 93–125.

Ziuganov, V.V., Arctic and Southern Freshwater Pearl Mussel *Margaritifera margaritifera* with Long and Short Life Span as a Model System for Testing Longevity Mechanisms, *Uspekhi Gerontologii*, 2004, no. 14, pp. 21–30.

Ziuganov, V.V. and Kalyuzhin, S.M., The Problem of Parasitic and Tumor Diseases in Atlantic Salmon *Salmo salar* in the Northwestern Region, *Ob"ed. Nauch. Zh.*, 2004, no. 8, pp. 63–70.

Ziuganov, V., Zotin, A., Nezlin, L., and Tretiakov, V., *The Freshwater Pearl Mussels and Their Relationships with Salmonid Fish*, Moscow: VNIRO, 1994.

Ziuganov, V., Beletsky, V., and Neves, R., *The Recreational Fishery for Atlantic Salmon and the Ecology of Salmon and Pearl Mussels in the Varzuga River Northwest Russia*, Blacksburg: Virginia Tech., 1998.

Ziuganov, V., San Miguel, E., Neves, R.J., and Longa, A., Life Span Variation of the Freshwater Pearl Shell: A Model Species for Testing Longevity Mechanisms in Animals, *Ambio*, 2000, vol. 29, no. 2, pp. 102–105.

Ziuganov, V., Popkovitch, E., Beletsky, V., and Neves, R., Tolerance to Starvation by the Long-lived Freshwater Pearl Mussel *Margaritifera margaritifera* (Bivalvia, Margaritiferidae), in *Abstracts. World Congr. Malac. Unitas Malacologica*, Salvini-Plawen, L. and Voltzow, J., Eds., 2001a, p. 405.

Ziuganov, V., Kaliuzhin, S., Beletsky, V., and Popkovitch, E., The Pearl Mussel-Salmon Community in the Varzuga R., Northwest Russia: Problems of Environmental Impacts, *Ecol. Stud.*, 2001b, vol. 145, pp. 359–366.