

I. ABSTRACT.

Until recent decades, Antarctic resources were regarded to belong to no one and, therefore, were harvested without restrictions. This overexploitation ultimately led to the near-complete exhaustion of economically valuable Antarctic animals. The process began with the largest whale species, followed by seals, smaller whale species, porpoises, penguins, large species of icefish and now krill (57). At present further exploitation is planned and administered (2) sustainable fishery has to take into account the amount of fish, which needs to be based on estimations of all age groups present. Fishing in the past was only focusing on large icefish specimens (57) and did not provide ichthyologists with sufficient information on the size and distribution of younger, smaller Antarctic fish and did also not provide the means for making age and growth estimates for which icefish otoliths of all age groups would have been necessary (37; 23), something, however, that is difficult to obtain (75; 43; 71; 23). Mucha, in his publication on the stock of icefish, *Ps. georgianus*, emphasized that the age data he had used, contained unavoidable errors (48). Finally, determining the age groups of Antarctic fish from descriptions of their occurrences has been (and still is) difficult to researchers throughout the year and especially during the Antarctic winter, because of the inaccessibility of the fish's habitat of sharp rocky peaks and the considerable depths that the fish occur in.

Therefore, this doctoral dissertation's research aim was to examine the microstructure of otoliths (64) to enable the determination of age groups and life histories from it. A very broad, multifaceted approach to this issue depended on analyses of the regularity of the elementary increase of the microstructure of the otoliths (62; 64) in relation to the fish's growth and the changes in otolith shapes during ontogenesis (62). These results were used to assess the age structure of the fish (63) and their abundance/biomass dynamics and regional occurrences (63), thereby facilitating our understanding of interspecies connections (61; 24) and their relationships with optimal habitats (63; 61; 24). Differences in different icefish species and age groups were recorded (62; 64) and related to cyclical seasonal changes (63; 61) and food competition. This was a new approach for understanding the problems that were created by the commercial exploitation of Antarctic icefishes. To do so, I needed to consider the entire range of fish lengths, including the smallest 7-cm, age-group 0 individuals that also appear in the commercial fishery to estimate catch limits and true growth parameters. This included determining the lengths and ages of all individuals including those that have the same or very similar species-specific adult body shapes and share the same environment. The relationships that I determined, provided a picture of the strong inter-dependencies between developmental biology (63), ecology (61), seasonal periodicity (63), and climate change (61).

A detailed knowledge of otolith microstructure is the basis for more precise and accurate icefish age estimation (64). These fish have no other structures that are useful for age assessment (23) because they lack scales, and other bony elements are reduced or absent (75) and, therefore, not suitable for age assessment (71). However, historically, interpretation of icefish otolith microstructures in relation to annual increments was difficult and without satisfactory results (23; 43; 71). Also, the otoliths of *Pseudochaenichthys georgianus* (henceforth abbreviated *Ps. georgianus*) are very brittle, light and fracture easily, hindering age analyses (62; 43). Otolith of *Chaenocephalus aceratus* (henceforth abbreviated *Chaen. aceratus*) were lighter than *Ps. georgianus*, (62) they

contain more proteins that are creating the macrostructure, because of which they need removing aragonite from 2 sides of the otolith section while for *Ps. georgianus* from 1 side only to see the collagen structure of the daily increments. Otoliths of *Champscephalus gunnari* (henceforth abbreviated *Champ. gunnari*) were lighter and smaller than *Chaen. aceratus* and *Ps. georgianus* (64). Age of *Champ. gunnari* (57) were estimated from analyses of frequency of total length over long time series (personal communications with Józef Sosiński). Age groups of *Chaen. aceratus* (2) were estimated from the number of hills (= projections) at the otolith edge (personal communications with A. Kompowski). In otoliths of icefish, seasonal increments were discovered (51) that overstated 2-4-times the estimation of the age group in response to the lack of distinctions from year-old increases. Mucha noted that *Ps. georgianus* age groups, read from otolith microstructures, contained errors (48) and therefore refrained from age group estimates. The interpretation of annual otolith increments performed by specialists from around the world provided very different estimates of the age groups in the range of 1 to 17 years with an error of 8 ys for the same otolith (11). Therefore, I performed detailed studies of *Ps. georgianus*, *Chaen. aceratus* and *Champ. gunnari* otolith microstructures to determine how and why they form and to relate particular patterns and composition to growth, age groups, and life histories.

The elements of otolith microstructure that constitute the smallest period of otolith growth are tropocollagens, 300 - 280 nm length (72; 25) aggregated at the same time each day into a surface network of diamond-shaped mesh fibres and needles of crystallised aragonite (52; 64; 72). This cannot occur in reverse (72). During periods of low motor activity, maximum protein synthesis by *pars intercerebralis* neurosecretory cells occurs (17). The daily deposits of crystallised aragonite needles (52) show cross-sections on otoliths of narrow rings of tropocollagen fibres on the sides of rhombic (rhomboids) and wider rings of rhombic meshes filled with aragonite needles, increasing concentrically from the otolith centre to the edge (30; 64; 53). Because the thickness of the collagenous fibre constitutes narrow rings, they have a solid width, which remains invariable and restricts the size of aragonite. In contrast to them wide rings, because the rhombus-eyes of the collagenous net that constructs them, they change the width of the daily rings through the change of the arrangement of the rhombus-eyes, leading to an expansion and squeezing.

Otoliths play important roles in the perception of body balance (62; 64), responding to changes in body position, detecting hydrostatic pressure and perception of sound waves. These functions are reflected in their microstructures (74; 47) and relative concentrations of aragonite and proteins (collagen, conchiolin and spongin). Thus, environmental phenomena are responsible for the arrangement of the otolith microstructure. The otoliths congregate from the dipoles of dissolved tropocollagens, arranged and concentrated in the endolymphatic waves of acoustic condensations and dilutions (46), appropriate to dipole conformations.

Thus, otolith mesh widths differ with age and activity. Daily larval otolith surface growth is 0.0009 mm (62; 64). On the dorsal growth axis of the *Ps. georgianus* otolith, the mesh width averaged 0.0019 mm, after larval about 45 daily deposits *. In comparison with otoliths' daily increments of young *Chaen. aceratus*, *Champ. gunnari* and *Ps. georgianus*, the averages were 0.0016 mm, 0.0024 mm and 0.0028 mm respectively (64). In the slowest larvae, i.e. *Ps. georgianus*, swimming inshore towards calm bays, increments were as narrow as 0.0009 mm, but they were 0.0028 mm for faster young swimming in deeper, bottom outshore water of 400 m depth and with 0.0034 mm fastest for the adults * that swim in strong (8) surface currents and vortices *. Those

different increment widths result from pressure changes on the growing surface of the otolith driven by the increased size and speed of the developing fish (72; 64). Icefish of the older developmental stages have larger swim speeds (8). The larvae of these icefish stay in strong surface currents, undergoing in them dispersal in the open ocean beyond their shelf habitats. However, the fish of older developmental stage migrate into these currents of neighbouring islands for food procurement (61). Daily increments arranged from the same length elements of tropocollagen (260 nm) should be similar. Changes between developmental stages and interspecies changes in the width of otolith increments and otolith shape result from different swimming strategies under different habitat conditions, changing with developmental stage (64; 60). Young *Ps. georgianus* make wide vertical migration to deep-water (51) for feeding on krill, which increases the width of the dorsal otolith increments and creates an additional growth centre from that deposited as a larva (62). This makes otoliths higher than longer (64). On the other hand, mature individuals swimming faster and feeding on larger krill at shallower depths, conduct horizontal migrations; their otoliths add an additional growth centre and lengthen (62). Such changes in otolith shape reflect changes in swimming speed and feeding strategy as well as increase their directional accuracy (64), which are important for resisting strong currents and increasing krill-capture success rates.

I used materials and data from ichthyologic and oceanographic measurements collected during international scientific expeditions recorded and controlled by the international scientific committee, of the Commission for the Conservation of Antarctic Marine Living Resources CCAMLR, from 1975 to 2007 (63; 61). Over 350 *Ps. georgianus* otoliths from fish ranging from 6-56 cm caught in the Antarctic summer from 1986 to 1990 were measured, weighed and tested against microstructure analyses. The external morphology of 1712 otoliths were measured and related to body morphology changes with individual development (62; 64).

Ps. georgianus is a nocturnal feeder, living in a dark environment during the day at a depth of 470 m. At night, during the decrease of luminosity in the water and when icefish recognize the sounds of krill (33), they migrate to a depth of 200 m. Current vortices are vertical channels (19) that carry acoustic information about the activity of krill accumulated in them and that focus *Ps. georgianus* foraging on them (64). The strategy of diurnal vertical migrations is reflected in the *Ps. georgianus* otolith shape, which has a greater height and thus better induces vertical deviations (64). However, this shape changes and becomes elongated for older horizontally migrating fish caught at the continental slope but spawning inshore along the coast (64).

Champ. gunnari retains the round larval otolith shape during development into the pelagic lifestyle, for which round otoliths are more suitable as balance detectors with faster pelagic swimming due to different adaptations to a pelagic life (64). Demersal *Chaen. aceratus* have large, more elongated otoliths similar to those of the grenadier, *Macrourus carinatus*, which control horizontal deviation more quickly when swimming at the bottom and feeding on large benthic animals (64).

Regarding *Ps. georgianus* near Palmer Archipelago, where winter ice cover is extensive and larvae and small krill are found, the fish were smaller (16 to 34 cm), whereas on the South Orkney shelf with its large krill population, only larger fish (33 cm to 60 cm) were present (63; 64). Off the South Georgia shelf, without ice cover, *Ps. georgianus* ranged from 7 to 60 cm. On the other hand, where 20 and 33 cm age-group 1 and 2 *Ps. georgianus* occurred, small krill from the Antarctic

Peninsula aggregated in the warm Antarctic Circumpolar Current. But older, larger *Ps. georgianus* were concentrated and migrated vertically in the vortex regions that concentrated large krill from the South Orkney Islands of the colder Weddell Sea (64). The biggest icefish concentrated at the shelf edge (64) where the krill of the open sea was. In addition to the recorded vertical migrations (51; 64), horizontal migrations were indicated by Mucha (48), that means there is a single genetic population with the same growth rate and distribution of age groups. The islands of the Antarctic Peninsula can be assumed to be a single habitat of several dispersed islands (63; 61; 48) connected by currents, underwater mountains and rocks, corresponding to the habitat of South Georgia maintained by the surrounding ocean (63; 61). Differences in fish lengths and otolith mass between climatic zones resulted from shifting development stages with climate change. Together, these data indicate that the spawning and hatching of *Ps. georgianus* occurs 2-3 months later in the Antarctic zone than on the South Georgia shelf.

Taking into account the unique biology of white-blooded fish, it should be emphasized that they all obtain 90% of the required oxygen thanks to the diffusion of oxygen particles from the sea into the blood through the skin and transport them in the plasma in a dissolved state, as none of them have actively transporting red blood cells. Dissolved oxygen transport provides only 10% of active transport in a bound state by haemoglobin. White-blooded fish, thanks to various adaptations resulting from the difference of their habitats, increase the diffusion of oxygen particles from the water into the blood. Therefore, the lack of scales in the skin of white-blooded fish is commonly translated as one of the general adaptations that increase the diffusion of oxygen through the skin into the body (15) in order to provide better heart function (37). According to Jakubowski (37), this is a widespread error. The researcher pointed out that the network of subepidermal capillaries is above the scales, so the scales do not separate the osmosis process from the oxygen dissolved in the ocean. The question then arises as to why white-blooded fish have lost their scales. The absence of scales increases the perception and use of water flow on a smoother body surface when swimming in sea currents at different angles, supporting the function of otoliths. The importance of such a function of the skin is confirmed by the development of as many as 3 lateral lines in *Ps. georgianus* - a species that swims and feeds in the strong currents of the Antarctic Current, its tall body (64; 26) allowing it to use the hydrodynamic energy of sea currents, and by the fact that all species of white-blooded fish have 2 or 3 lateral lines. In addition, greater smoothing of the reverse flows increases the flow velocities of water and oxygen with respect to a smoother streamlined skin surface, which increases the oxygen pressure gradient across the capillary walls and its diffusion accordingly. There is no proper explanation whatsoever for the loss of scales generally and icefishes in particular as we do not have the appropriate physiological results from fish studies and cannot assess this phenomenon in the past.

White-blooded fish divide the inhabited different habitats between different species and different stages of development, and thus ensure the best use of the small resources that limit life in harsh environmental conditions, for fish larvae (51), young fry, adults (63) and throughout the generations (61). Such a strategy necessary to survive, determined the adaptation of species of white-blooded and red-blooded fish (67) to separate temporally and spatially into adjacent habitats, expressed by different features and adaptations specific to a particular species, development stage, age group and, accordingly, a given habitat (62) and behaviour such as pelagic and demersal, coastal and open sea. In all species, diffusion passes through the thin walls of subepidermal capillaries of the body skin, fins, pharynx and gills, composed of a single layer of cells (37). Additionally, adaptations,

regulatory peptides involved in ionic homeostasis of body fluids and osmosis in *Champ. gunnari* gills were discovered (37).

The icy waters of Antarctica are rich in oxygen, its high concentration in the ocean increases its pressure gradient on the capillary walls, which thereby increases the diffusion of oxygen from high in the ocean to low concentration in the plasma of the capillary network, then transported to the tissues inside the body, and consumed in metabolic processes (differently for each species - depending on its more or less cold habitat). Deep waters and eddies cooled by the currents of the Weddell Sea concentrate *Ps. georgianus* (61) and therefore its blood is protected from freezing by the highest activity of anti-freeze proteins (*). The lowest activity of these proteins has been noted in pelagic *Champ. gunnari* (found in the warmer waters far to the north, on the Kerguelen Island shelf). The diffusion of oxygen from the water into the skin through the skin is increased by its very large surface area (8; 54) on the large head part, on the large fins to a varying extent. It is most pronounced in the cold-loving, semipelagic *Ps. georgianus*, in which the head part is 44% of the standard body length (SL) and body height is 23% SL; for bottom species, tolerant of somewhat warmer water as in *Chaen. aceratus*, the head part occupies as much as 40% SL and body height is 20% SL, but the lowest values are found for pelagic *Champ. gunnari* in which the head part is 31% SL and the height of the body is 17% SL (8; 37). In order to increase the transport of dissolved oxygen, white-blooded fish have more vessels and capillaries with larger cross-sections and larger blood sinuses, thanks to which they contain 2-4 times more blood volume, about 9% of body mass (37), containing more oxygen from the ocean water (37). Transport through larger cross-sections of the vessels reduces resistance (15; 35) and accelerates the flow of a larger mass of plasma and delivers dissolved oxygen to the tissues. Increasing the transport of dissolved oxygen by a greater flow in this transport, of large amounts of blood through the voluminous capillaries of gill plates and skin increases oxygen diffusion from seawater, but also increases the energy consumption of this transport carried out by the heart. While 5% of the heart is sufficient for red blooded species, white blooded fish use 22% for this purpose.

The realization of this consumption to increase the heart capacity varies between species: greater pumping in *Chaen. aceratus* is possible thanks to the special spongy structure of the heart muscle, which increases its size by 3 times compared to the same 320 g of trout heart mass, which at the same time absorbs more oxygen directly from the blood (38). In *Ps. georgianus* more oxygen for the work of the heart is needed and it is provided by the content of oxygen-storing myoglobin in the muscles of the heart (37), which generally increases diffusion by 600% (8; 18; 37); while a greater supply of oxygen to the heart muscles in *Champ. gunnari* is provided by increased blood volume, high stroke volume and cardiac output (heart rate product = number of beats per minute (HR) and stroke volume (SV) during ventricular systole [litre per minute]), large diameters of muscle capillaries and a high oxygen gradient between blood vessels and organs, which enables the maintenance of normal metabolic functions (41), while cardiac regulatory peptides are involved in cardiac homeostasis (67). The unique appearance of the parietal pericardium indicates a significant adaptation of the circulatory function of this animal. This species occurs in warmer pelagic waters, which allows it to have a higher water warming tolerance (8; 22), as well as a different composition of the heart membranes, which determines that it is less sensitive to temperature changes compared to *Chaen. aceratus* - the ability to maintain heart function at elevated temperatures (8; 22).

The lack of myoglobin in skeletal muscles in white-blooded fish limits the activity of muscle locomotion due to lack of oxygen, and determines the implementation of the type of energy-saving swimming: e.g., the pectoral fin swimming strategy (69; 65). It is most noticeable in *Chaen. aceratus* with its reduction of the axial musculature of the body and its weak vascularization. A decrease in body musculature must lead to a decrease in body mass, as the Channichthyidae do not have a swim bladder. Channichthyidae reduce the mass of the skeleton by replacing it with cartilage. *Chaen. aceratus*, increases swimming speed through a streamlined, grenadier-like body shape and larger body dimensions, enabling more energy to be supplied through more energetic food: large fish and large bottom animals. In *Ps. georgianus*, the swimming speed is increased by the high body structure, a species which uses the energy to swim in the sea currents, implementing the migration of development stages, feeding in the whirlpools and food migration against the current to the neighbouring island (61). Greater activity of the body muscles in *Champ. gunnari*, a species also swimming in warmer surface waters, is ensured by the lack of reduction of the axial muscles, their structure being similar to the muscles of fast-swimming salmonids and the greater density and diameter of the capillaries contained in them (37) and large spaces supplying the capillaries with blood (69). This leads to an increased oxygen transport in the muscles, which is not found in *Chaen. aceratus* (3; 37). Dense vascularization in the muscles reduces the diffusion distance of oxygen, increases oxygen transport.

Energy is also saved by increasing the size of the body or the diameters of the muscle cells. *Chaen. aceratus*' are the largest: larger sizes reduce energy losses through a smaller body surface area in relation to the volume. Likewise, with a low fibre area-to-volume ratio, less energy is used to maintain the electrochemical gradient of cell membranes with fewer coarse fibres than with more thin fibres, while muscle mass remains the same (58). When increasing the size of the body to lower the energy consumption for moving a greater mass of the skeleton and the body in swimming activity, we observe a reduction in body mass: axial muscles, bone skeleton, inhibition of ossification (most pronounced in bottom *Chaen. aceratus*, the least in pelagic *Champ. gunnari* individuals, which are smaller (26) and have numerous muscles of smaller diameter (8; 58). The reduction in body weight reflects a reduction in the weight of the otoliths corresponding to the function of the perception of movement of the body. is less saturated with aragonite crystals. Despite these adaptations, the activity and respiratory metabolism of clearly demersal *Chaen. aceratus* fish are the lowest, almost half of those of the semipelagic *Ps. georgianus* (37) occurring periodically at the bottom further away from the coast. And it is also lower than in the pelagic *Champ. gunnari* - the significant slenderness of the body of this fish species characterizes it as a fast-swimming fish, and that means high metabolic activity for them. The final conclusion of the biology of white blood fishes is that they cannot be treated together as identical or even similar species, because at different levels of metabolism there are no similarities in either the histological characteristics of the muscle cells or the shapes of their bodies (58; 8; 64; 61) or their distribution on the shelf and their life strategies (61) or other biological characteristics that are marked by the microstructure and shape of their otoliths. However, they should be compared together to better understand their different biologies.

Icefish fulfil their ecological roles within substantial temporal and spatial temperature oscillations (61). In cold years and periods, *Ps. georgianus* is the most numerous species (61), concentrated in cold currents, whereas in warmer years *Chaen. aceratus* is dominant because it is

less dependent on low temperatures and on ice-related krill, creating an inter-species biological buffer (61; 57; 42).

The adaptations of species in the 'icefish family' start from the sequence of hatching of their larvae and not from a competition for food (51). Larvae of *Ps. georgianus* after earlier hatching inshore in cold winter weather rapidly migrate to colder deeper water below 300 m (64) for fast growth, safe from warming (70). However after 3 years of growth and gonad development in the cold and deep water, they return to the inshore habitat for reproduction (64) that results in large future biomass increases in cold years, but decreases in warm years (63). The biomass of the semipelagic *Ps. georgianus* oscillates like that of krill with a 4-year periodicity of life stages (63) in contrast to the temperature of cold – warm year cycles (61) and to the coexisting bottom icefish *Chaen. aceratus*: the former with a lower biomass in warm years and a higher one in cold years (61). Larvae of *Chaen. aceratus* after later inshore hatching at the end of winter and warm spring have a lengthened pelagic development in summer, but slowly migrate to permanently cold bottom water to change their food intake from krill to bottom animals (8). They do not rely on the lack of krill in warm years and do not compete with *Ps. georgianus* for krill.

Larvae of *Champ. gunnari* are the latest to hatch in the warm spring and early summer and then undergo a lengthy development in the warm pelagic environment throughout all years to becoming adults, relying almost 100% on krill as food in (42). Its biomass also oscillates, but the species tolerates warmer water with its lower activity of antifreeze proteins (8). Spending a longer period in the upper pelagic waters, it avoids to compete with semipelagic and bottom specimens of the other two species. The result is that the biomass of all three species considered together is rather stable (61).

In view of their adaptation to temperature changes, reduced Antarctic icefish stocks (63) seem to be driven most by decreased krill availability (61; 42), which alters the feeding strategy of krill predators (61; 64; 42). In addition, mass and size of individual krill decrease in current warmer waters because it is the ice-associated species growing optimally at cold temperatures that disappear because of global warming (70; 8). For many months the Southern Ocean is a huge ice-cold water desert, devoid of ice cover because of global heating and thus devoid of ice algae that are the food of krill feeding under the ice (55; 49; 70). Such water deserts create insufficient prey for *Ps. georgianus* and other krillivores, which generates strong food competition and increased pressure of large predators on fish (24).

As icefish fisheries increased, *Ps. georgianus* stocks decreased (61; 64). Despite bans on catching them, the species did not recover, but actually trended downward (63). A stock assessment of *Ps. georgianus* indicated a biomass declination by about 75% less than virgin resources (63; 61). While its level was $36 \cdot 10^3$ tons in 1976/1977, it dropped to $3 \cdot 10^3$ t in 2004/2005 (63). In the recent year 2021, bycatch was below $1 \cdot y^{-1}$ ton (13). An analysis of the species contribution in the biomass of all fish (13; 63; 61) showed a large oscillation from $2 \cdot 10^3$ to $10 \cdot 10^3$ tons, indicative of a decrease.

A similar reduction with large oscillations is also exhibited by the target catch species *Champ. gunnari*. While the biomass of *Champ. gunnari* was about $106 \cdot 10^3$ tons in 2014 (56), it dropped to $10 \cdot 10^3$ t in 2021 (56). For both species a lack of large fish specimens was noted. *Ps. georgianus* average body length decreased from 50 cm to 38 cm (63; 61), while *Champ. gunnari*'s domination decreased from peaks of around 36 cm to 10 – 20 cm total length (56). Not even one fish

above 46 cm was reported, but up to 50 cm TL fish were frequently caught in the 1975-1977 seasons (56). There is no definitive answer as to why the large biomass of white-blooded fish did not recover despite some decades of securing and reducing their catch (57). *Ps. georgianus*' abundance is closely related to the density and distribution of krill, which is the basic food of both young and adult fish (33; 8). The occurrence of *Ps. georgianus* strongly modifies the catch of krill. As the krill yield increased, the stocks of *Ps. georgianus* decreased (61; 64). These resources, despite the ban on catching them, do not recover, but actually show a downward trend (63). This is because high krill catches are maintained every day throughout the year (13). Also the lack of an ice cover increases fishing of krill, which in case of feeding under the ice cover, is not available for fishery.

The continuous presence of krill throughout and between the Scotia Arc island shelves, recorded in the 1970s and 1980s (55), was accompanied by a homogeneous distribution of about 100% krillivorous *Champ. gunnari* across the shelf, with no noteworthy high school densities noted (13). In later years, in those krill continua, there appear huge areas lacking an ice coverage and associated krill - that is, so-called water deserts caused by the elimination of the ice cover by the warmer ocean water (the southern retraction of its northern border) and increased by constant fishery of krill, by economic law focused on large density shoals. When the number of krill decreased and the consumption of ice algae under the ice by krill disappear, this contributed to the formation of pelagic krill shoals that fed on pelagic plankton instead individuals on ice algae (55; 61). The ice cover in the 1980s winter can reach South Georgia Island, generates high biomass of ice algae and krill feeds on it under the ice up to 13 km to south with high densities about 30,000 individuals per cubic meter (55). In summer when ice algae flourish density is equal to 10^6 cells per cubic meter, krill development (feeding on the abundant algae) reach up to 13 million to some billions tons a year (55). Lack from warming and disharmony in the cover of ice, cause the disappearance of half of the food source for krill while the decline of krill density increases day by day by krill fishery focusing on large densities of krill, thereby constantly decreasing krill availability as food. Aggregations and shoals of krill alternating with water deserts can be deemed a determining factor in the corresponding change of the organizational level of the switch of pelagic *Champ. gunnari* to previously unregistered high-density pelagic shoals (57), the latter having a greater chance of encountering krill shoals in the pelagic desert, leading to more successful foraging (55). The water deserts between krill shoals reduce the krill's continuous availability and the success of *Ps. georgianus*' vertical overnight migration to krill foraging at the depth of its occurrence at night, about 200 m (64). Therefore, semi-pelagic *Ps. georgianus* also change their strategy (to different degrees due to body shape and biology) and focus on the sites of the Weddell-Scotia Confluence vortices accumulating krill (61) at the north-eastern shelf of S. Georgia (55). Bottom dwelling *Chaen. aceratus* changed their life strategy the most or retained a permanently demersal life with increased swimming speed and changes in food from krill to demersal animals, becoming independent of the pelagic krill resource (8). Therefore, in more frequent and longer water deserts, where the numbers of large *Champ. gunnari* and *Ps. georgianus* fish have been declining since the 1980s, the most likely cause would be the reduced availability of krill food, affecting, however, large bottom *Chaen. aceratus* less severely (13). Reductions of the density of krill and increasing areas and time periods of water deserts reduces the abundance of fish of the studied species and other krillivorous animals (icefish, whales, penguins), in favour of increasing numbers of fish that do not consume krill: such as *Gobio notothen gibberifrons*, *Macrourus carinatus*. As a part of being their food, krill also make and constitute a favourable environment for larvae and young of fish including icefish (13; 61; 33). Changes in the

occurrence and abundance of pelagic plankton in Antarctica has been observed (55; 49; 70). The shelter for larvae, food for larvae and a well-oxygenated habitat friendly to living larvae and the success of icefish reproduction are affected (33).

When presenting the effects of contaminants present in Antarctica (73) on white-blooded fish, their unique biology must be taken into account. The fact that none of them have red blood cells and all get 90% of the oxygen they need from the diffusion of its molecules through the skin has the consequence that pollutants such as dioxins penetrate the skin completely unhindered as oxygen molecules and affect their biology. Adaptations to the increase in oxygen diffusion increase the diffusion of dioxins, poisonous compounds that living organisms recognize as oxygen molecules. In Antarctic waters, dioxins from untreated waste of 4,000 people at research stations from 40 countries accumulated in bottom sediments at levels as high as those recorded for the Baltic Sea (7). They are the main type of pollutants due to the long period of lack of control, various breakdowns, the lack of treatment plants (45) and the reduced possibility of deactivation due to low temperatures. Antarctic stations have been operating for decades, especially those rebuilt from whaling settlements. In their vicinity, in the bottom sediments, the wastes from these years from these stations constantly poison aquatic animals, especially white-blooded fish, every year. Since 1790, i.e. from the beginning of whaling exploration, harmful compounds have accumulated on the seabed and accumulated in biota, especially near Antarctic research stations (45; 12; 31; 73). This is due to disturbances caused by numerous fishing vessels (6) and - more and more often - also in connection with tourism, as well as due to the transport of adsorbed dioxins on carriers of the ubiquitous microplastic material with sea winds and currents from distant sources (44). All this could have contributed to the decline in the number of large white-blooded fish in terms of their length, as well as limiting the range of the species.

Microplastic alone, as up to 5 mm particles of ubiquitous polymers imitating food eaten by pelagic and benthic animals causes their death and as a far-reaching carrier of adsorbed dioxins with them poisons distant environments of the Southern Ocean. The lipophilic properties and low saturated vapor pressure result in the sorption of dioxins on apolar surfaces of fine inorganic particles, on fly ash and on organic particles suspended in the air, and with them spreading over long distances (5). Some dioxins themselves are also very capable of traveling long distances with wind, rain and ocean currents, and have been found near poles where they have not been used (44). Due to the transport of dioxins to the poles, their pollution can be assumed from the very beginning of their creation by man. Since 1953, during the industrial revolution, many cases of dioxin leakage outside closed technological circuits have occurred in various chemical plants around the world (40). Due to the accumulation of previous years, the dispersion of dioxins by atmospheric and water erosion may be greater, although the current pollution with them could be at a low level. The emission of dioxins used in high concentrations from the sludge 20 years ago in high concentrations was demonstrated (66). So they lasted in the sediments for 20 years. Dioxins in water absorb on the surface of particles of substances suspended in water and sink with them to the bottom, where, due to poor biodegradation, they accumulate in bottom and sewage sediments. Dioxins are very persistent and thermodynamically stable. These substances are resistant to biochemical changes and hydrolysis - they are not biodegradable, because of their low solubility in water makes it difficult for them to be degraded by microorganisms. They are temperature resistant. Dioxins are rapidly absorbed by diatoms, which are eaten by krill, and then by white-blooded fish and other krillivorous animals, which causes their bioaccumulation (16) in the fatty tissues of these animals. When

consumed, dioxins are not metabolized, they remain in the body, accumulating in it with any food containing dioxins. Dioxins are also found in the stool of krill as well as in krill processing waste that sinks to the seabed near human research sites and stations. There, a high level of dioxins in bottom sediments was recorded (7; 16). Fish and animals living close to the Antarctic continent, and therefore close to stations inhabited by humans, show greater bioaccumulation of dioxins in the body for bottom-dwelling species than for pelagic species (68). The species of benthic animals, having high levels of dioxins, also indicate high pollution of bottom sediments at Antarctic stations (7; 16).

Dioxins are small molecules that are two benzene rings, linked by two or one oxygen atoms, forming the central ring of dioxin or furan, having one to all replacements of hydrogen atoms on high chemical activity and electronegativity halogens: chlorine, bromine, iodine, fluorine in unmixed or mixed proportions: polychlorinated (-brominated, iodinated, fluorinated) dibenzo-para-dioxins / furans (PCDD / F, PBDD / F, PJDD / F, PFDD / F,..., etc); dioxin-like molecules (polychlorinated biphenyls, PCBs, diphenyl ethers, PCDE, naphthalenes, PCN); molecules of polycyclic aromatic compounds as well as molecules containing sulphur instead of oxygen in their structure, i.e. polychlorinated thiantrenes and dibenzothiophenes. Fat-soluble, small, flat, nonpolar dioxin molecules can jam anywhere and replace substrates; accumulate in skin lipids (10). They easily cross the cell membranes of the capillary wall because they dissolve in the hydrophobic, non-polar part of the lipid cell membrane. Then some dioxin molecules leave the lipid membrane towards their lower concentration inside the cell. As a result, molecules pass from the ocean through the capillary wall cell membrane (s) into plasma, like oxygen molecules. White blooded fish can therefore absorb dioxins through their skin and bioaccumulate them through the consumption of other fish in their diet (21; 20). When assessing the extent to which dioxins affect white-blooded fish, it is worth considering the nature of the habitat of a particular family species in the context of the fact that dioxins, unlike oxygen, accumulate in bottom sediments, so benthic species will experience the greatest destructive effects in the biology and occurrence, and semipelagic and pelagic species may have smaller ones. This is confirmed by the research results. A greater risk of dioxins occurs in white-blooded fish, especially bottom fish. The pelagic organisms of white-blooded fish, such as *Champ. gunnari*, have a 40 times higher concentration of pollutants than the pelagic organisms of *Gymnoscopelus nicholsi* (9). The red-blooded fish *G. nicholsi* and *Electrona carlsbergi* showed no destructive effects, no abnormal otoliths were found in the studies, contrary to the frequent abnormalities observed in otoliths of white-blooded fish such as *Ps. georgianus*, *Chaen. aceratus*, *Chionodraco hamatus*. *Chaenodraco wilsoni* (64). The shape, composition and microstructure of otoliths is a feature that characterizes the species.

Dioxins acting in a double, enhanced way, as they affect white-blooded fish by giving them, like oxygen molecules, free access to the interior through the surface of the entire body, causing mutagenic changes, they affect the structure of the genetic code in a way that leads to degeneration in the next generations. The action of dioxins is slow, manifesting itself sometimes only after a dozen or several dozen or more years. The long-term effects of even low doses of dioxins may be more dangerous than their acute toxicity (10). By acting teratogenic and embryotoxic, they damage reproductive materials. They are a first class carcinogen (36): 400,000 Vietnamese died and 500,000 Vietnamese children were born handicapped and underdeveloped due to the effects of dioxin used in the 10 years of the Vietnam War (29). In addition, it was found that in the dioxin-exposed areas of Vietnam the number of bird species decreased sevenfold and that of mammals 10 times (14). The

same was the case with the abundance of white-blooded fish in the Antarctic zone. During the scientific expedition of the rv "Profesor Siedlecki" in the area of South Orkney, not a single adult *Ps. georgianus* and not a single larva of this species in the 1989 season were caught, while in this area during the virgin stock years they were present in high numbers. Also in the catch of fish larvae in those years of the species *Ps. georgianus*. By binding to plasma lipids and lipoproteins, dioxins are transported through the bloodstream to tissues and organs of the entire body (10). Dioxins generate genetic changes and weaken various processes (66). In biological processes of self-organization of the spatial arrangement of substrates in relation to hydrophobic and lipophilic groups, they change structures (10), i.e. they disturb the processes of self-organization, and such a process is the formation of otoliths.

Recently, abnormal otoliths have been observed, reducing their mass and shape (loss of edges, replacement of aragonite with lighter vaterite). The collection of otoliths of white-blooded fish, published in 1987, does not mention any abnormal otoliths, while abnormal otoliths are now described in numerous publications. There are deficiencies in the current layers of growth of the entire edges of the otoliths, which are debris, or their dissolution, seen in *Ps. georgianus* or continued gaps started in the past in *Chion. hamatus* and *Ps. georgianus* (64), not previously observed. There are also substitutions of aragonite for vaterite in white-blooded fish in the Antarctic zone, which has not been observed before. These changes may indicate the observed changes in the reduction of ossification forming cartilage up to bone deficiency, which may result in reduced acoustic perception due to the absence / change of echo-eliminating bone conduction (46). Bone deficiencies may even cause asymmetries of the two-sided symmetry of the body in *Chaenodraco wilsoni* found in the Antarctic zone, where there are most research stations. The two-sided symmetry of the body is the most important evolutionary achievement for the success of any species at a higher level of evolution. Therefore, deficiencies and defects in bone development causing defects in acoustic perception and asymmetry rather correspond to the effects and nature of dioxin activity. It is difficult to accept here the goal of the evolutionary process, eliminating the lack of a swim bladder (75), in view of the potential creating a wide variety of biology of white-blooded fish. Also, the deficiencies in the growth of the otolith edge may be the result of the influence of dioxins, the more so as they do not occur in young fish, but appear in older fish (64), which migrate to reproduce and lay eggs on the bottom to the coastal zone where dioxins are deposited. It may also be an adaptation to changes in body weight and centre of gravity occurring annually in large fish during the production of large spawn mass (64). On the other hand, the reduction of the otolith mass by forming the collagen macrostructure of otoliths in *Chaen. aceratus* (64), may correspond to the reduction of ossification in this species, explained by adaptation to low temperatures by reducing energy consumption for reduced body mass in species of benthic white-blood fish (37). The activity and respiratory metabolism of clearly demersal *Chion. Hamatus* fish have the lowest activity, despite the adaptation of increased oxygen diffusion and transport.

Monitoring of dioxin concentration is difficult and costly. The established methodology for determining the concentration of dioxins in bottom sediments requires protection against the toxic effects of volatile, liquid and solid compounds during the sampling at sea and the determination of dioxins in the laboratory, which must have accreditation certificates (50). Dioxins are a sub-micronutrient component of bottom sediments, concentrations are <1 ppt (<10-8%), determined by column chromatography. Thus, cheaper, safer methods of analyzing the natural signs of otoliths resulting from the influence of dioxins on biology and the presence of white-blooded fish are needed

to reduce the risk of destruction of the bones of the skull and skeleton. The altered otoliths are manifested by disturbances in the concentric growth pattern of the otolith microstructure (64) and lead to reduced acoustic perception of the environment (39). Such altered otoliths become unsuitable for age estimation of fish (64).

Determining icefish age groups should be based on the daily pattern of concentric rings, otolith microstructure, and statistical constants, because this ring pattern is permanently recorded and the same in larval (53; 62; 64) and adult (62; 64) parts of the otolith. Furthermore, they can be determined and verified with tests for the smallest constant periodicity (64; 32) and they can be replaced by the constant mass increment of the otoliths (64). Thus, it is not necessary to know the various changes in seasonal or annual signs in otoliths to estimate age groups from daily increments in otolith microstructure (23; 43; 71; 11; 48; 64)

The species-specific shape of the otolith (34) shows the dependence of this shape on their life strategies. This underlines the importance of the daily variability in physical activity (17). Furthermore, information from otolith age readings not only reveals the fish's age groups, but it also shows their relationships with the environment (63; 61). Lastly, icefish aging offers insights into the persistent 75% decline in *Ps. georgianus* abundance (13).

My research provided a comparative analysis of the changes affecting *Ps. georgianus* otolith microstructure, shape, and mass. Those changes were compared with those of other icefishes, such as *Champ. gunnari* and *Chaen. aceratus*; species leading different lifestyles and colonising different environments during their development, which accounts for otolith changes relative to the ontogenetic and phylogenetic development (64).

In my research, I also used selected material from the CCAMLR Scientific Commission, which underlines the high scientific value of international research work and cooperation. Biological studies complementing climate monitoring are recommended by the Climate Convention to better understand the ecological effects of climate change (4). Such studies will facilitate predicting and mitigating unfavourable changes, including 90% biomass reductions of large-mass biota, such as whales, seals, birds, icefish, and krill *, body-size reductions of Antarctic organisms (13; 61) and reduced genetic variation. The unknown extent and direction of changes in icefish biology associated with global warming can be approximated by tracing the effect in relation to differences between cold Antarctic waters versus warmer South Georgia shelf waters (63). In addition, the distribution of Antarctic research stations creates pollution patterns in shelf habitats and their biota (12). Nonetheless, recent changes in icefish life-strategies indicate their capacity of adapting to environmental changes (75), and even pollution effects and that gives us some degree of hope for their future (57).