



SPATIAL ORIENTATION OF FISH EMBRYOS IN MAGNETIC FIELD

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Abstract: Orientation, i.e., spatial arrangement, of fish embryos in artificially generated (with constant magnets) 0.5; 1.0; 2.0, and 4.0 mT horizontal magnetic fields, superimposed on the geomagnetic field was compared to the orientation in the Earth's magnetic field alone (control). In addition, effects of artificially generated 0.5 mT magnetic field superimposed on the geomagnetic field, but rotated by 90° relative to it, were followed. Fish eggs were incubated in a constant, unchanging position, until the blastomere closed, and the orientation of the embryo was determined. The embryos showed statistically significant preferences towards the N-S orientation of their axis of symmetry. In addition, the artificially generated constant magnetic fields were found to induce significantly stronger orientation responses, compared to those elicited by the geomagnetic field alone.

Key words: fish embryos, orientation in magnetic field

Introduction

The Earth's magnetic field is a permanent component of the natural environment and provides a backdrop to life processes on the planet. Over the recent decades, much attention has been paid to spatial orientation of animals and their utilisation of the geomagnetic field in migrations (Keeton 1971; Gould 1980; Quinn and Brannon 1982; Lohmann et al. 1995).

Numerous studies dealt with migratory fish. The European eel (*Anguilla anguilla*) were demonstrated to be able to return, over far distances, to their native areas (Deelder and Tesch 1970). Observations on the non-migratory *Anguilla anguilla* showed the fish to exhibit preferences towards the North and the South. During migrations, the eels preferred the North orientation. On the other hand, no such preferences were exhibited by the juvenile eel, the elvers (Tesch and Lelek 1973). The geomagnetic field can be also used in orientation and navigation by the American eel (*Anguilla rostrata*) (Rommel and McClave 1973; McClave and Power

1978). In addition, both *A. anguilla* and *A. rostrata* showed a relationship between the magnetic field and salinity vs. direction of movement (Tesch 1974). A change in the position of the magnetic N resulted in a corresponding switch in the direction of migration of the European eel (*A. anguilla*) (Tesch et al. 1992). Behaviour of the migrating adult chum salmon (*Oncorhynchus keta*) off Hokkaido, subjected to a modification of the circa-cephalic magnetic field (Yano et al. 1996) was studied as well. Research described by Walker (1984) showed the yellowfin tuna (*Thunnus albacores*) to be capable of recognising different magnetic stimuli.

Experiments on orientation of the Chondrichthyes (sharks and rays) in magnetic fields showed the presence of directional responses to the field direction and an ability of the fish to accordingly align the body in the natural environment (Kalmijn 1982).

The presence of a specific directional response in the fish was detected during studies, carried out under natural conditions, on the performance of the fyke

nets, i.e., fish traps, rigged with special sets of constant magnets. Those studies confirmed the directional response in the fish species caught, as the magnet-rigged fyke nets attracted more adult (Formicki et al. 2001, 2002, 2004 b).

Research on the directional response in juvenile chum salmon (*Oncorhynchus keta*) showed the fish, when placed in special tanks, to move in the direction of their natural migration pathway (Quinn and Groot 1983). Similarly, the experiment reported by Quinn and Brannon (1982), involving smolts of the sockeye salmon (*Oncorhynchus nerka*) demonstrated the fish to be capable of exercising orientation in the magnetic field. The fish, placed in circular tanks in the normal geomagnetic field, turned towards the river's discharge from the lake they had been caught in. Both adults and the 6-month-old juveniles of the rainbow trout (*Oncorhynchus mykiss*) showed preferred directions in the natural magnetic field (Chew and Brown 1989). Studies on the larvae of sea trout (*Salmo trutta*) offered a selection of the movement direction showed the young trout to most frequently move towards the weak, constant, artificially generated magnetic field (Sadowski et al. 2003, Formicki et al. 2004 a).

So far, it has been possible to demonstrate that both constant and alternating magnetic fields affected numerous physiological processes in early ontogeny of fish. The effects are manifested in, i.a., the history and duration of the embryonic development, embryonic and larval circulation, water and gas exchange, and enhanced fertilisation (Perkowski and Formicki 1997; Formicki et al. 1998; Formicki and Winnicki 1998)

Preliminary studies on orientation, i.e., arrangement of the axis of symmetry of the body, in embryos of the sea trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) placed in magnetic fields (Formicki et al. 1997; Formicki and Tański 2000) prompted the authors to address the question if the phenomenon observed is

typical only of migratory species that wander over long distances, or – on the contrary – it occurs also in those species that do not undertake such migrations, or are regarded as non-migratory.

Materials and methods

The study was carried out within 1998-2002 in aquariums kept in controlled temperature rooms at Department of Fish Anatomy and Embryology, Agricultural University of Szczecin (Szczecin, Poland), in the Department's field laboratory on Lake Krzemień (Izdebno near Sieraków Wlkp., Poland), and at the Jeziorno hatchery operated by the Fish Farm at Lutom near Sieraków Wlkp.

The experiments involved developing eggs of salmon (*Salmo salar* L.), sea trout (*Salmo trutta* L), rainbow trout (*Oncorhynchus mykiss* (Walb.)), vendace (*Coregonus albula* (L.)), pike (*Esox lucius* L.), and rudd (*Scardinius erythrophthalmus* (L.)).

Gametes obtained from each individual of each species were transported, at a temperature identical to that in the spawning ground, from the respective fishing grounds to the laboratory separately in 0.75 l thermos bottles (eggs) and in glass vials (sperm) placed in thermos bottles. The thermos bottles were additionally protected by placing them in 20 dm³ insulated containers equipped with cooled inserts maintaining the low temperature desired. At the laboratory, to select the best material possible, the eggs and sperm were subjected to a preliminary qualitative evaluation; a mixture of eggs obtained from 5 females was fertilised by sperm from 6-7 males (the so-called dry fertilisation).

The fertilised eggs were incubated in 3 dm³ cylindrical opaque mini-aquariums treated as orientation arenas. The eggs were placed on a plastic netting that served as a substratum. The eggs developed at species-optimal constant temperatures.

The netting was extended with circular supports 3 cm above the bottom of the

aquarium. The water level above the netting was 3 cm. The netting mesh size was adjusted to the egg size, whereby the eggs could not change their position. The relatively large amount of water around each egg ensured adequate oxygen conditions. In addition, one-third of the water in each aquarium was changed every other day during incubation of eggs of salmon, sea trout, rainbow trout, and vendace, daily in the case of pike eggs, and every 3 h in the case of rudd. The water was changed simultaneously in all the aquariums which were connected, with silicone tubes, with a master tank containing constantly aerated water, previously left to stand, of a temperature identical to that in the aquariums. The tubes were equipped with valves ensuring the water change rate of $0.5 \text{ dm}^3 \cdot \text{h}^{-1}$. Care was taken not to change the height of the water column above the netting (the amount of inflowing water was equal to the outflow). The very slow, partial substitution of the water did not alter the position of the developing eggs throughout the period of incubation.

The incubation proceeded in a darkened room, to rule out all visual orientation cues, and at a constant temperature, appropriate for each species: 8°C for salmon, sea trout, and rainbow trout; 7°C for vendace; and 17°C for rudd.

Horizontal magnetic field was generated by ferritic constant magnets equipped with expanders, placed on both sides of each mini-aquarium. This made it possible to generate a constant magnetic field affecting the developing embryos throughout the experiment. The developing eggs were incubated in 0.5; 1.0; 2.0, and 4.0 mT constant magnetic fields, superimposed on and directed identically as the local geomagnetic field. Another experimental treatment involved application of an 0.5 mT magnetic field rotated by 90° relative to the background geomagnetic field. Some mini-aquariums were subjected to the geomagnetic field only and constituted a control.

The magnetic field value was point-measured with a HTM-12 hallotronic teslameter (Institute of Telecommunications and Acoustics, Wrocław Technical University, Poland).

Spatial arrangement of each developing embryo was assessed after the closure of the blastopore, when the embryos are well-visible but immobile, i.e., after 135 D° in the case of salmon; after 120 D° in sea trout; after 110 D° in rainbow trout; after 65 D° in vendace; after 51 D° in pike; and after 290 h° in rudd. Assessing the embryo's spatial arrangement at a later phase of the development, when the embryos are mobile, could have been biased. The embryo's position was determined using a 16-rhumb scale (wind rose) enabling a precise recording of the position of each embryo relative to a magnetic field lines.

The data were subjected to a statistical treatment involving Rayleigh's test (Baschelet 1981).

Results

The salmon embryos placed in the 0.5; 1.0; 2.0; and 4.0 mT magnetic fields as well as in the geomagnetic field (control) adopted, in a statistically significant manner, mainly the N-S orientation (Figure 1A-E).

Similar was the spatial arrangement of the embryos of sea trout (Fig 2A-E) and rainbow trout (Figure 3A-E).

The axes of symmetry of the vendace embryos placed in the 0.5; 1.0; and 4.0 mT magnetic fields as well as in the control showed a significant preference towards the N-S orientation, other orientations being observed in the 2.0 mT field (Figure 4A-E).

The pike embryos subjected to the 0.5; 1.0; 4.0 mT revealed a significant preference towards the N-S orientation, no significant preference being visible in the 2.0 mT field and in the control (geomagnetic field) (Figure 5A-E).

No statistically significant preference towards any particular orientation of the

axis of symmetry was displayed by the rudd embryos developing in the 0.5 mT field; on the other hand, those embryos incubated at the 1.0; 2.0; and 4.0 mT and in the control showed a significant preference towards the N-S orientation (Figure 6A-E)

The 90° clockwise orientation of the magnetic field (0.5 mT) lines, relative to those of the geomagnetic field, resulted in the embryos' axes of symmetry alignment with the lines of the artificial magnetic field stronger than that of the Earth.

The developing salmon embryos preferred mainly the N-S orientation (Figure 7A). A similar trend was observed in the developing embryos of sea trout (Figure 7B), rainbow trout (Figure 7C), vendace (Figure 7D), pike (Figure 7E), and rudd (Figure 7F).

Discussion

Directional preferences in the arrangement of the embryonic axis of symmetry in the artificial constant magnetic fields were displayed by the embryos of all the fish species tested in this study. As the preferences were exhibited not only by the migratory species, it cannot be contended that the embryonic orientation is in any way related to the orientation ability of adults in the geomagnetic field.

Artificial magnetic fields of various intensity were observed to cause certain differences in deviation of the embryonic axes of symmetry from the major N-S direction. Moreover, the 2.0 mT magnetic field induced no significant axis of symmetry directionality in the developing embryos of pike and vendace, nor did the 0.5 mT field cause such directionality in the developing embryos of rudd, although such directionality did occur in the remaining magnetic field intensities tested. Cleary (1993) described effects of magnetic fields on biological structures in the system of the so-called windows. The phenomenon involves susceptibility of an organism to effects of certain magnetic

field parameters. Although the author quoted ascribed the "windows effect" to the electromagnetic field, it is highly probable that a similar effect might be associated with constant magnetic fields.

Rotation of magnetic field poles in the artificial 0.5 mT field induced the embryos to align their axes of symmetry to the artificial field lines. This confirms the decisive effect of the artificially generated field on embryonic orientation, relative to the weaker natural geomagnetic field.

The spatial orientation of fish embryos, found in this study, could have been a result of a magnetic material produced by many fish species. That material may be regarded as responsible for fish orientation, as the presence of magnetite was found in, i.a., the head of the yellowfin tuna (*Thunnus albacares*) (Walker et al. 1982), in the body of chinook salmon (*Oncorhynchus tshawytscha*) and chum salmon (*Oncorhynchus keta*) (Kirschvink et al. 1985; Ogura et al. 1992), in the cranium of European eel (*Anguilla anguilla*) (Hanson et al. 1984) and in the lateral line of salmon (*Salmo salar*) (Potter and Moore 1991). However, it does not seem probable that the magnetic material controls the selection of the embryonic axis of symmetry direction during the embryonic phase tested, i.e., from fertilisation (initial divisions) to the closure of the blastopore (termination of gastrulation), because magnetite appears in the body most likely as a product of metabolic activity proceeding throughout the life span of an individual, hence its higher amounts in the adult fish (Chew and Brown 1989; Kirschvink et al. 1985). Assuming that magnetite production begins early in the embryonic life, the amount of the magnetic material present is then very low and it is most probably not used by an embryo which does not move actively until the blastopore is closed and the nervous system has not formed yet.

It has been difficult to explain the mechanism of animal orientation in the magnetic field, because it is hard to

identify structures responsible for the process (Kirschvink and Walker 1986; Hanson and Westerberg 1987; Lohmann and Lohmann 1996). Presumably, responses to magnetic fields is controlled by complex systems, as is the case in, e.g., in the rainbow trout olfactory epithelium that transmit signals under the influence of changes in magnetic field intensity (Walker et al. 1997).

When trying to elucidate the mechanism likely to control embryonic orientation in the geomagnetic and artificially generated fields, attention should be paid to the embryonic movements that take place as of the very beginning of embryogenesis. The movements were described by Korzelecka (1999) who used the term quasi-peristalsis to characterise movement of ectoplasm at early ontogenetic stages, whereby the waves of protoplasm moved meridionally and laterally over the yolk sphere surface (Rezničenko 1965; Winnicki et al. 1998; Tański et al. 2000). Those characteristic movements were geared at making sure that the embryo develops under optimal oxygen conditions when the yolk material is used for building the embryo's body. Embryonic locomotion was observed in numerous fish species, including pike (Rezničenko 1965; Tański et al. 2000), three-spined stickleback (Winnicki et al. 1998), vendace, crucian carp, bream, and rudd and is species-specific, because the timing of the first movements and their duration differ between various species (Korzelecka 1999). Quasi-peristalsis in early embryogenesis has not been observed in some cyprinid species, but their ectoplasm does move, although not as intensively as, e.g., that of pike at the beginning of the embryonic development. This is perhaps why the pike embryos showed no significant preference towards the direction of body orientation. The lack of preference might have been related to the periblast movement at the initial phase of the embryonic development (Rezničenko 1965; Korzelecka 1999).

It may thus be contended that the directional quasi-peristalsis of the periblast at early stages of organogenesis as well as the meridional-lateral undulating movements of the protoplasm, correcting the alignment of the body, coupled with constant magnetic field effects on the magnetic material or paramagnetic particles help to explain the mechanism of embryonic orientation along the magnetic field lines.

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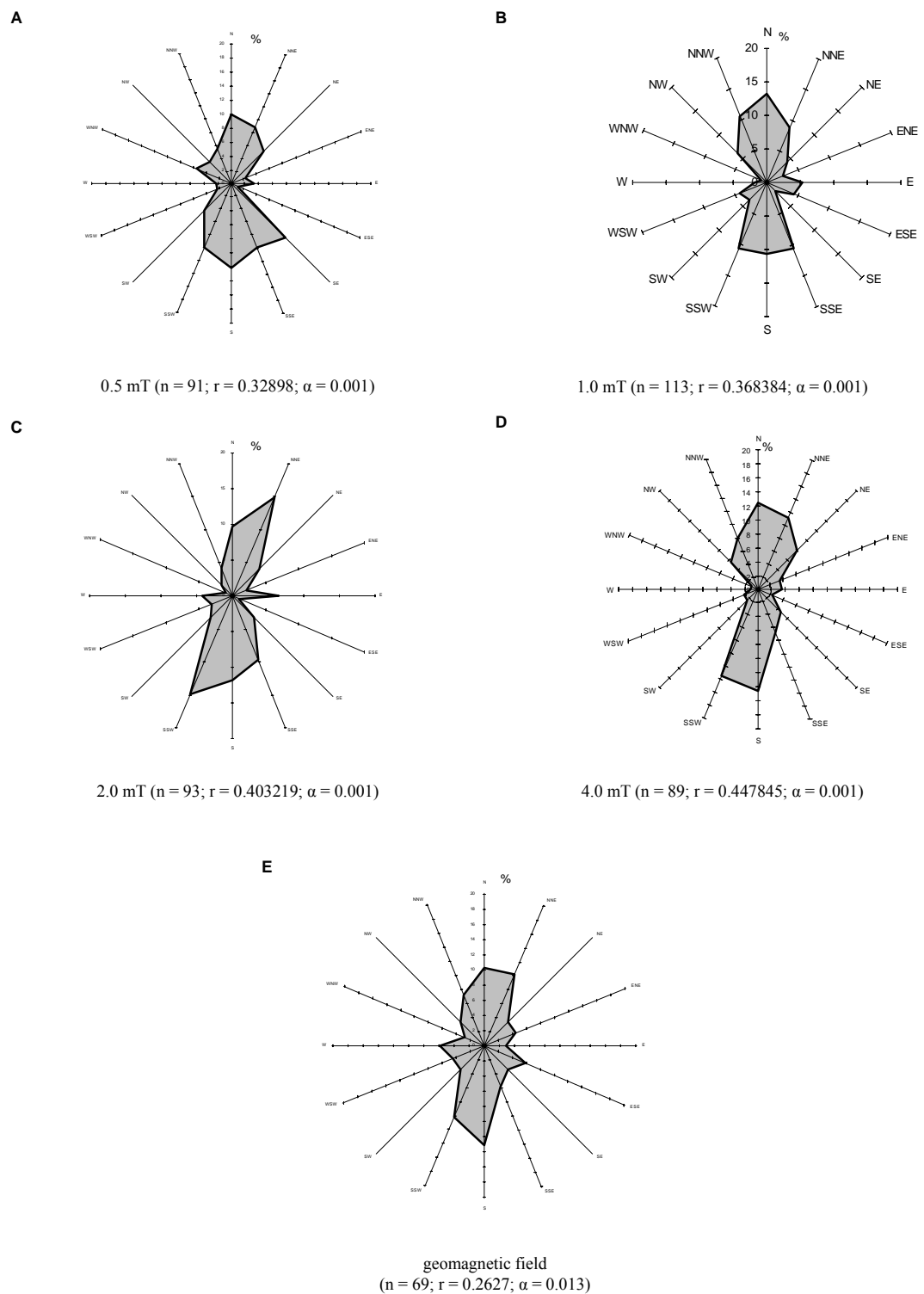


Figure 1. Spatial arrangement of salmon (*Salmo salar* L.) embryos in artificial constant and natural magnetic fields, as determined after 135 D°

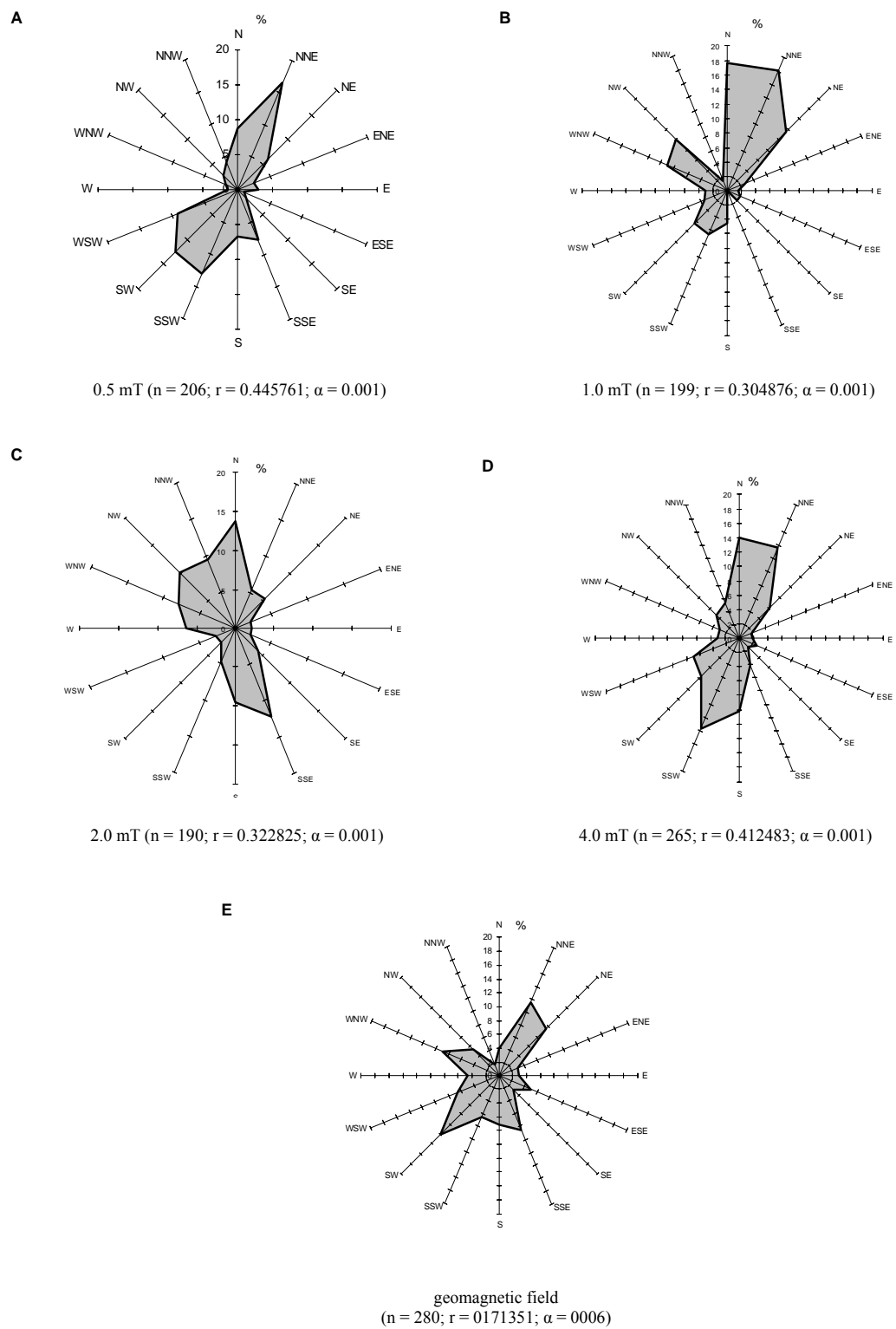


Figure 2. Spatial arrangement of sea trout (*Salmo trutta* L.) embryos in artificial constant and natural magnetic fields, as determined after 120 D°

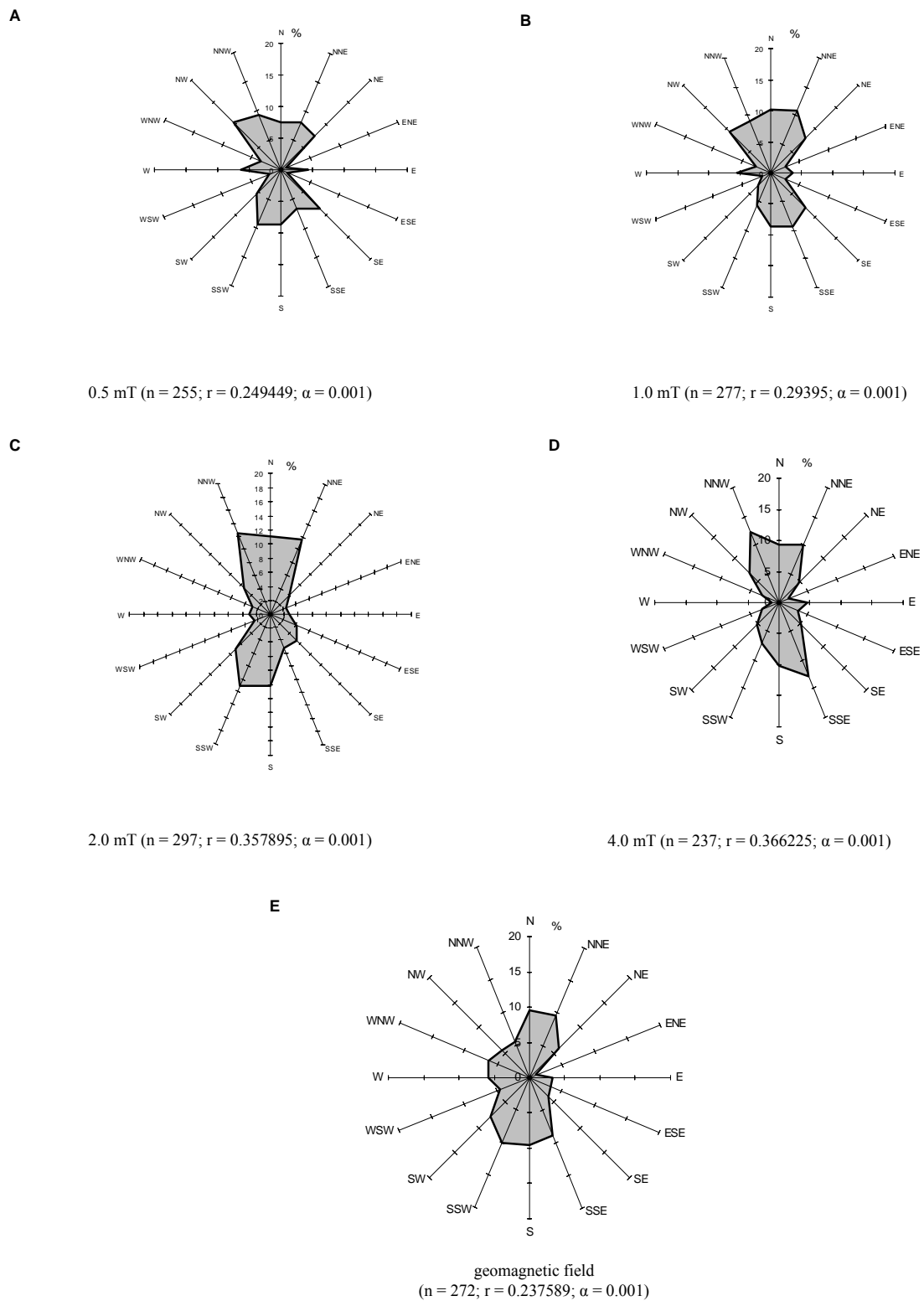


Figure 3. Spatial arrangement of rainbow trout (*Oncorhynchus mykiss* (Walb.)) embryos in artificial constant and natural magnetic fields, as determined after 110 D°

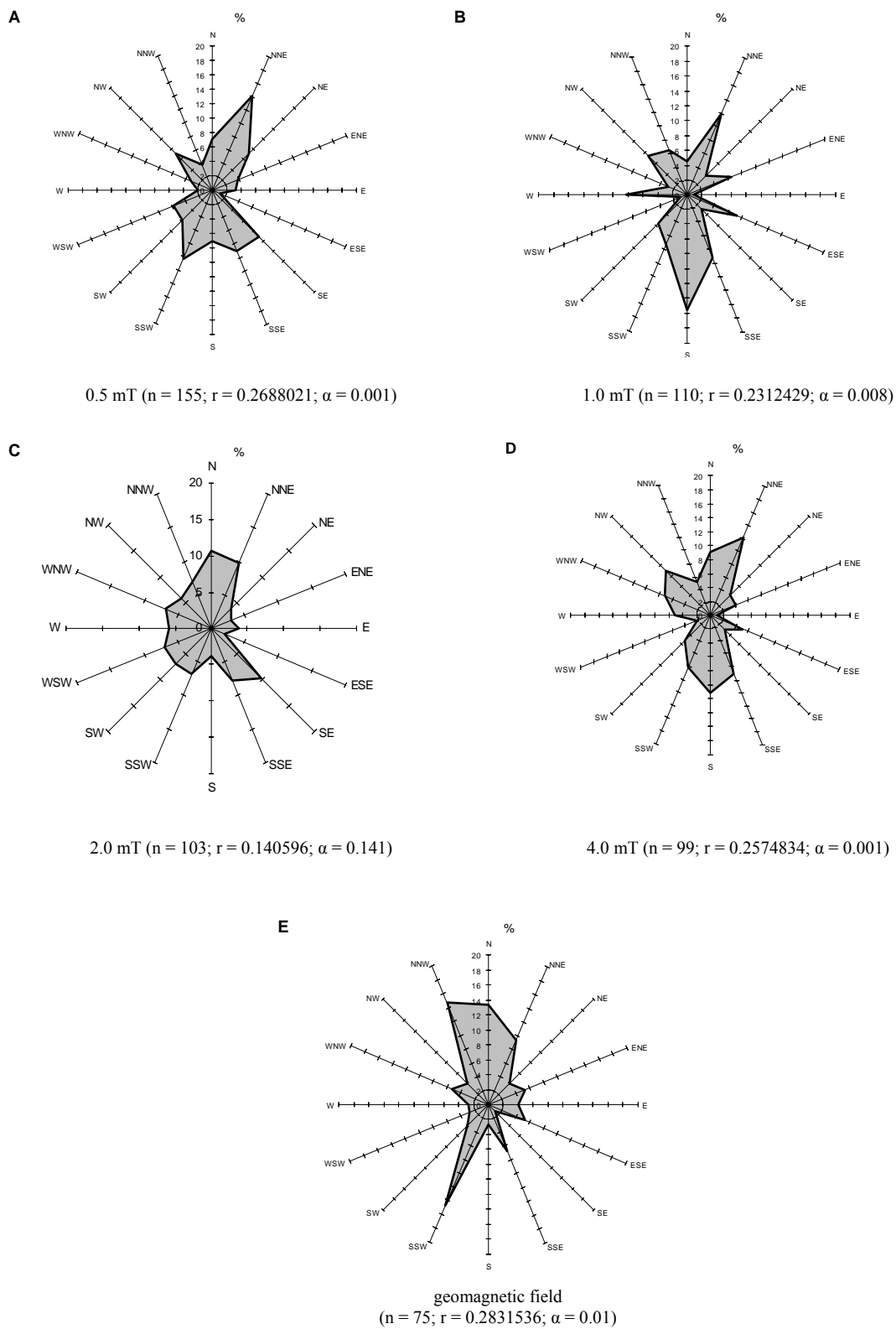


Figure 4. Spatial arrangement of vendace (*Coregonus albula* (L.)) embryos in artificial constant and natural magnetic fields, as determined after 65 D°

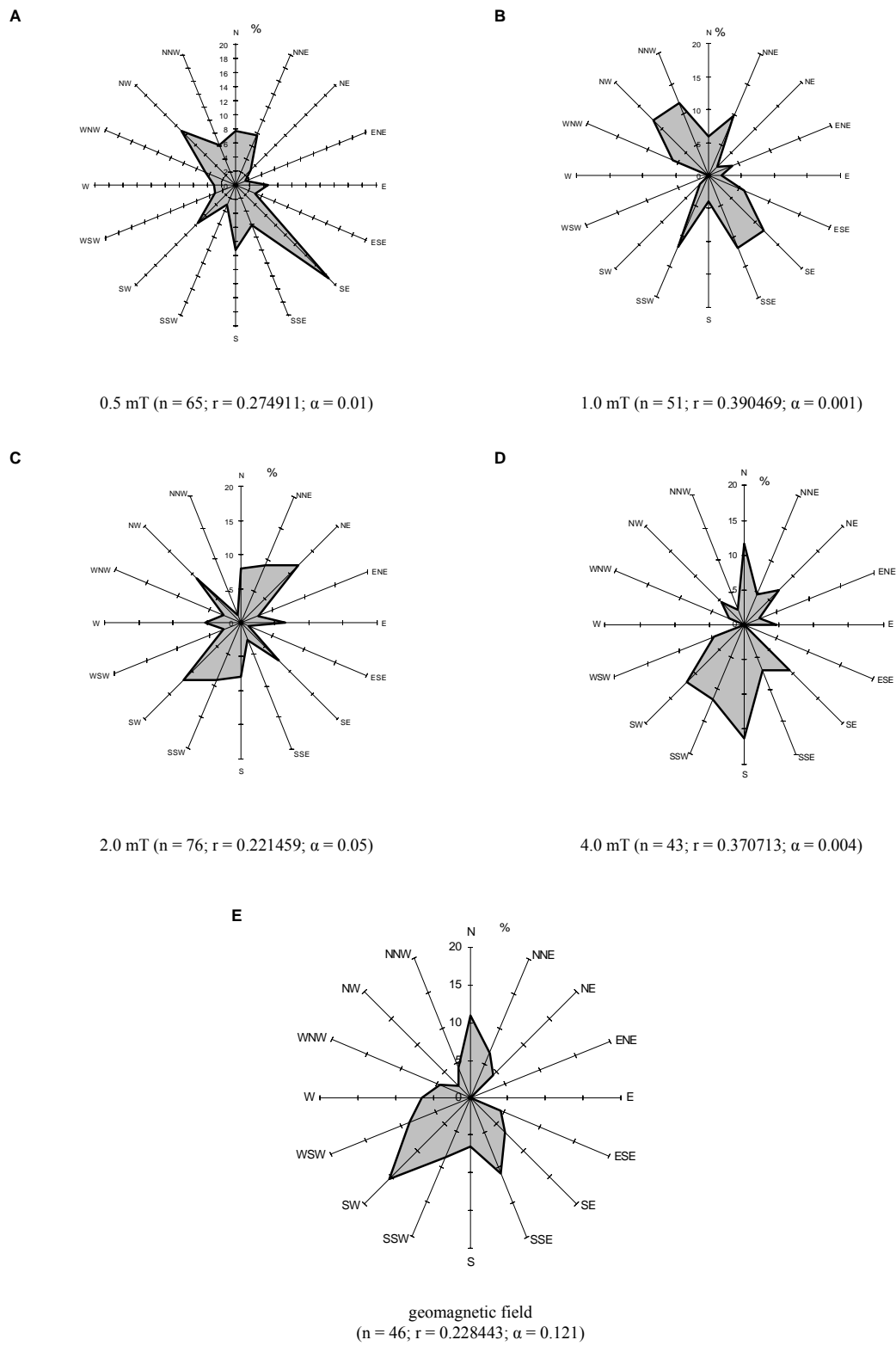


Figure 5. Spatial arrangement of pike (*Esox lucius* L.) embryos in artificial constant and natural magnetic fields, as determined after 51 D^o

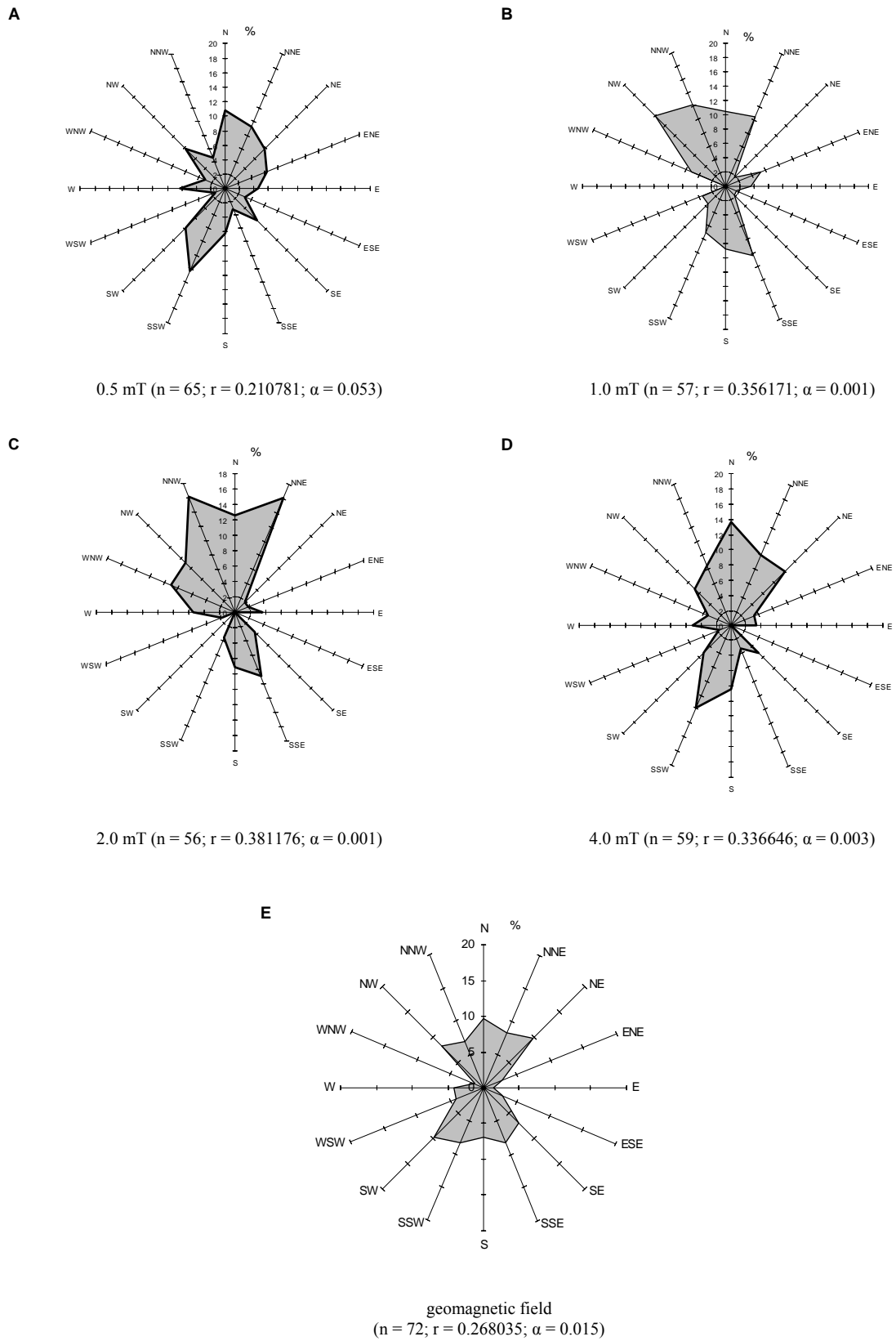
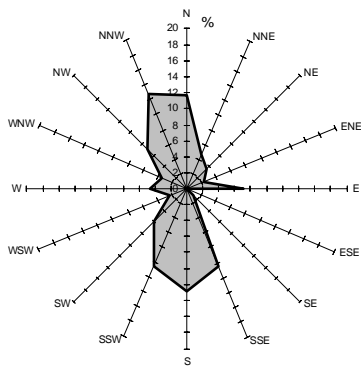


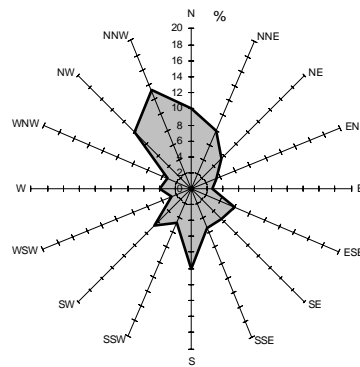
Figure 6. Spatial arrangement of rudd (*Scardinius erythrophthalmus* (L.)) embryos in artificial constant and natural magnetic fields, as determined after 290 H°

A



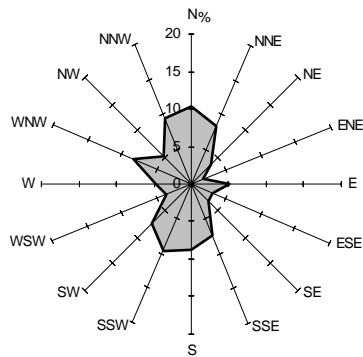
Salmon, 135 D° (n = 86; r = 0.343758; α = 0.001)

B



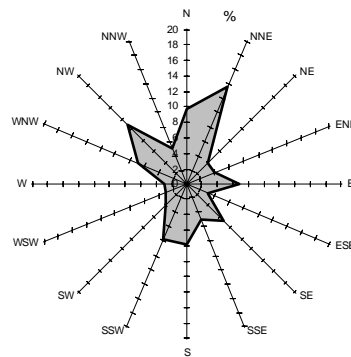
Trout, 120 D° (n = 151; r = 0.263973; α = 0.001)

C



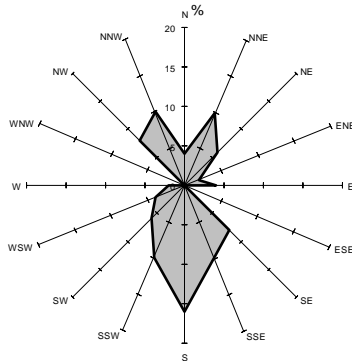
Rainbow trout, 110 D° (n = 298; r = 0.222185; α = 0.001)

D



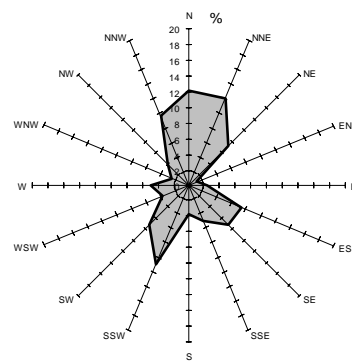
Vendace, 65 D° (n = 103; r = 0.184063; α = 0.039)

E



Pike, 51 D° (n = 50; r = 0.3804245; α = 0.001)

F



Rudd, 290 h° (n = 83; r = 0.253819; α = 0.037)

Figure 7. Spatial arrangement of fish embryos in artificial 0.5 mT constant magnetic field rotated clockwise by 90° relative to the geomagnetic field.