Chapter 6
Ichthyotherapy

Martin Grassberger and Ronald A. Sherman

6.1 Introduction

Ichthyotherapy is defined as the treatment of skin disease (so far mainly cornification disorders like psoriasis and ichthyosis) with the so-called "doctor fish of Kangal", *Garra rufa* (Heckel 1843). In accordance with other biotherapy terminologies, such as maggot therapy (use of sterile fly larvae, or "maggots"), hirudotherapy (use of the medicinal leech, *Hirudo medicinalis*) and apitherapy (use of the honeybee, *Apis mellifera*), the term "Ichthyotherapy" was proposed in 2006 (Grassberger and Hoch 2006) and readily adopted. The name derives from the Greek word for fish ("*Ichthys*"). Other descriptive terms frequently used are: "Dr. Fish treatment", "Kangal Fish therapy" or "Nibblefish therapy".

In recent years, many reports in the media featured this kind of treatment due to its apparent peculiarity. Wellness spas opened all over the world offering predominantly foot care for callus removal. "Fish foot therapy" was offered in recreational "fish spas", shopping malls, department stores and tea saloons in Japan, South Korea and Singapore, and later spread throughout Southeast Asia and all over the world. In 2008, the fish spa hype swept through the United States, with countless fish foot spas opening in many states. Due to the lack of regulation and industry standards regarding the treatment itself, the fish species used, humane treatment protections for the fish, and the absence of hygienic precautions for the clients, it did not take long until the first negative reports appeared in the media. Authorities soon

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took notice of this, sometimes fishy business. Consequently, numerous spas were shut down by the health authorities due to concerns over the potential for spreading communicable diseases as a result of customers sharing the same fish.

Notwithstanding, the publication of pilot clinical studies (Özcelik et al. 2000; Grassberger and Hoch 2006) and numerous anecdotal reports indicate that ichthyotherapy is a promising treatment for psoriasis, and deserves further study.

6.2 Historical Aspects

When news of the "Doctor Fish of Kangal" first came out of the central Anatolia region of Turkey, this alternative therapy must have seemed quite odd. The treatment was first mentioned in *The Lancet* in 1989 by Warwick and Warwick (1989) but details were not published until more than 10 years later by Özcelik et al. (2000) in the *Journal of Dermatology*. This Turkish study reported considerable benefits for patients suffering from psoriasis.

6.2.1 The Kangal Hot Springs

In the hot pools of Kangal, where food plankton is reportedly scarce, two fish species from the carp and minnow family feed on the skin scales of patients with illnesses such as psoriasis and atopic dermatitis (Timur et al. 1983; Özcelik et al. 2000). The two species are *Cyprinion macrostomus* and *Garra rufa*. The activity of the fish (especially *G. rufa*) is associated with reducing the scales and symptoms of these skin diseases (Fig. 6.1).

The Kangal Spa was built in 1900, but did not open to the public until 1963. It is located 98 km from Sivas and 13 km north of Kangal, Turkey, at an altitude of approximately 1,660 m above sea level. There are several pools with a mean temperature of 37 °C. According to the Kangal Spa authorities, it attracted the attention of the public in 1917, when a shepherd hurt his foot, "only to see it healed by the water of the spring".

6.2.2 Clinical Studies

Thus far, the medical and scientific communities have given ichthyotherapy very little attention. Treatment efficacy has been evaluated in only two published studies.

The first clinical study, published in the *Journal of Dermatology* by Özcelik et al. (2000), involved 87 patients from the Kangal Hot Spring Spa. The second study, published in 2006 by Grassberger and Hoch, was a retrospective analysis of 67 psoriasis patients who underwent 3 weeks of ichthyotherapy in an outpatient treatment facility in Austria.

Fig. 6.1 Patient seeking relief of his psoriasis symptoms in one of the pools at the Kangal hot spring (Photo courtesy of Dr. Wim Fleischmann)

At the *8th International Conference on Biotherapy*, Grassberger (2010) presented initial data from a questionnaire-based health related quality of life study in a cohort of 82 patients. To the best of our knowledge no additional clinical research on this promising treatment has been published.

6.3 The Reddish Suction Barbel – *Garra rufa*

Two different types of fish live in the pools of the Kangal hotspring: *Cyprinion macrostomus* and *Garra rufa*. Both fish are members of the carp and minnow family (Cyprinidae). *Garra rufa*, also called the "reddish log sucker" or "reddish suction barbel" is regarded as the primary therapeutic species. Because of its history of use in the Kangal hot springs and most of the ichthyotherapy facilities around the world, it is now regarded as the "gold standard" species for ichthyotherapy.

Garra rufa is a non-migratory freshwater fish found in rivers throughout much of Iraq, Israel, Jordan, Turkey, Syria, and possibly also Oman and Saudi Arabia (Berg 1949; Menon 1964; Abdoli 2000; Teimori 2006; Coad 2012). *Garra rufa* is found in a variety of habitats: rivers, lakes, ponds and muddy streams. This fish preferentially lives at the bottom of flowing rivers, where it adheres to rocks by suction using

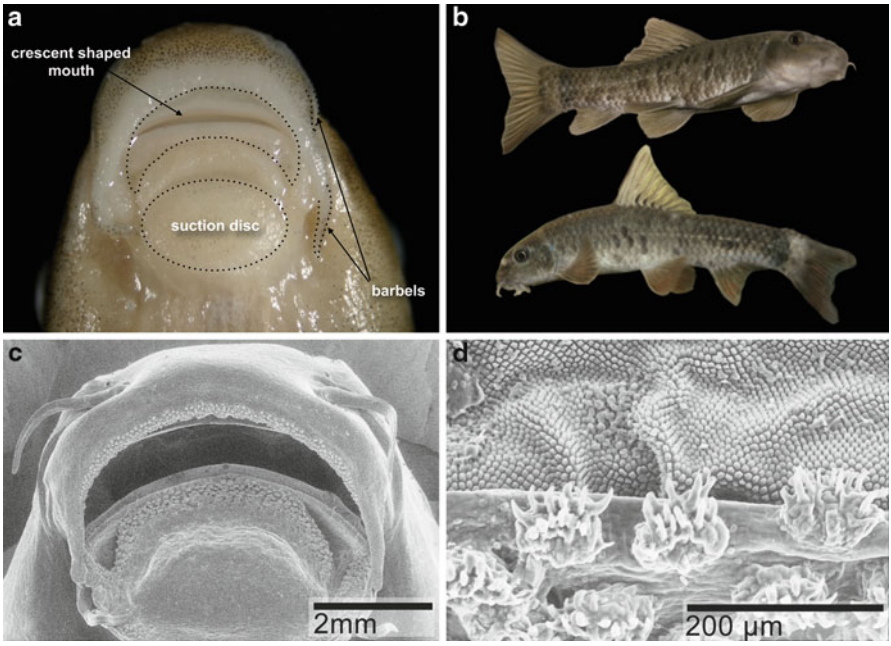

Fig. 6.2 Anatomy of *Garra rufa*. (**a**) Gross view of ventral crescent shaped mouth with suction disc and lateral barbels; (**b**) lateral and ventro-lateral view; (**c**, **d**) scanning electron microphotographs of the mouth with its surrounding tiny spines

its ventral crescent-shaped mouth (organ of attachment; Fig. 6.2a). Accordingly the head is depressed and bears two dorsolaterally placed eyes, resulting in an overall very distinct shape (Fig. 6.2b). It feeds on phyto- and possibly zooplankton.

As in other Cyprinidae, the barbels are slender, fleshy structures on the snout and chin, used for touch and taste. The crescent-shaped mouth of *G. rufa* has no external teeth (Fig. 6.2c). However, scanning electron microphotographs demonstrate a fringe of grouped minute spines (Fig. 6.2d), which appears to serve the fish as a scraper while feeding on outgrowths or human epidermis. The adhesive organ in this species is a very complex combination of integumentary modifications, as described in detail by Teimori et al. (2011).

There are currently about 180 species described in the Genus *Garra* that can be distinguished by morphological characteristics (www.fishbase.org; Kullander and Fang 2004). Whether any other species in this genus exhibit the same therapeutic potential as *G. rufa* is unknown, and could be an area for future research.

6.3.1 "Chin Chin Fish"

When photographs from various "fish foot therapy" spas were published, it became apparent that many of these new spas used fish species quite different from *G. rufa.*

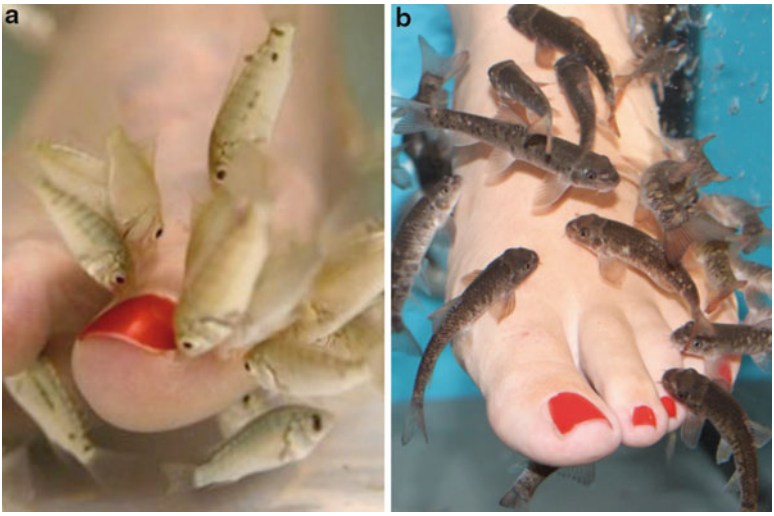

Fig. 6.3 Young Tilapia species, the so-called Chin Chin fish (**a**) in a fish foot spa compared to *Garra rufa* (**b**)

Most of these fish have been identified as juvenile *Tilapia* species (e.g. *Oreochromis niloticus*). When kept hungry, these fish will aggressively nibble on anything. However, no therapeutic properties have ever been associated with this species. As long as they are small (approx. 2–3 cm), they may look quite similar to *G. rufa* to the untrained eye. Nevertheless, closer observation reveals a strikingly different anatomy. Its mouth is not crescent-shaped and ventral as in *Garra* spp., but rather positioned apically, resulting in a completely different nibbling pattern (Fig. 6.3). As these fish grow larger, they feed more aggressively on the skin, and can be painful. They can cause skin wounds that act as portals for water- or fish-borne pathogens. Since they sometimes cause bleeding, they could cause human pathogens from one client to contaminate the water and infect another client. Without an effective water sanitation system, clients could develop severe soft tissue infections or could even acquire blood-born infectious.

6.4 Clinical Efficacy of Ichthyotherapy

In 2000, Özcelik et al. published their study of "Kangal Hot Spring with Fish" as a treatment for psoriasis. The study followed 87 patients (49.4 % male, 50.6 % female, mean age mean 32.5 ± 14.3 years) with psoriasis vulgaris. The mean length of stay in the spa was 11.5 ± 6.6 days. The patients used the pools twice daily and the mean length of stay of the patients in the pools was 7.4 ± 1.1 h in a day. Sixty-five patients (74.7 %) had plaque type psoriasis vulgaris and the mean duration of their symptoms was 10.6 ± 5 years. Fifty-two patients (59.7 %) in the study group came to the hot springs of Kangal for the first time, while 35 (40.3 %) had visited the hot springs previously.

The patients were evaluated by a dermatologist throughout the study, using the standardized Psoriasis Area and Severity Index (PASI score). The PASI is a physician-assessed score, recognized by the US Food and Drug Administration to assess efficacy of psoriasis therapies in clinical trials. The PASI score takes into account the extent of involved skin surface and the severity of erythema, desquamation, and plaque induration. The composite score ranges from 0 to 72, with higher numbers indicating more severe disease and a reduction in score representing improvement.

The researchers demonstrated significant improvement in psoriasis symptoms and PASI score within just 3 days ($P<0.01$). Follow-up at 21 days was available only for 14 patients, but was associated with complete and lasting benefits in 8 (57 %) of them. The remaining six patients had improvement in their symptoms, but not complete resolution. The 35 patients previously treated at the hot springs reported longer remission following their last spa treatment than they normally have following corticosteroid treatment.

According to the authors, many different factors may have contributed to the observed beneficial effects of spa therapy. Apart from the strikingly rapid clearing of the skin scales by the fish and the better penetration of ultraviolet light after removal of the scales, a positive psychological effect because of the initially rapid treatment progress was advocated. Additionally, the authors attributed the observed treatment effect in part to the high selenium level (1.3 mg/L) in the spring water. The only side effect reported was first-degree sunburn in two patients at the beginning of the therapy.

Interestingly, the authors described the fish as "attacking" areas with lesions, and also pointed out that the fish may "invade normal skin". These observations may be due to the fact that there are two fish species living in the Kangal Hot Springs: *Garra rufa* (referred to as "licker") and *Cyprinion macrostomus* (also called "striker"). *C. macrostomus*, being the more aggressive fish, may have been the cause of the small skin wounds. In the pools of the remote Kangal hot spring patients are required to take their bath with up to 20 patients simultaneously, which might be unacceptable for some patients and could be of concern for public health issues regarding the possible transmission of infectious disease.

In 2006, Grassberger and Hoch published the results of a retrospective analysis of 67 patients (39 male and 28 female) diagnosed with moderate to severe chronic plaque psoriasis who underwent 3 weeks of ichthyotherapy at an outpatient treatment facility in Austria. Patient's ages ranged between 10 and 75 years [mean 41 years, 95 % confidence interval (CI) 37.5, 44.5]. The mean duration of psoriasis at baseline was 13.9 years (range 1–35 years; 95 % CI 11.6, 16.2). All patients referred themselves to the treatment facility.

In contrast to the Kangal Hot Springs study (Özcelik et al. 2000), each patient completed a full 3-week treatment course. In addition, the daily "fish bath" lasted only 2 h, and each patient was allocated to a single bathing tub for the entire study period (no two patients shared the same tub at any time during the study). According to the authors this shortened daily treatment time probably made ichthyotherapy more acceptable for the patients and considerably improved compliance.

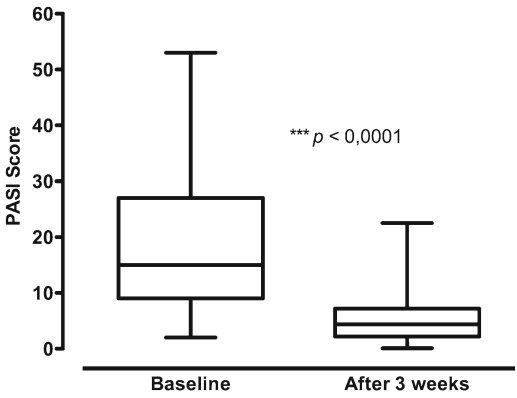

Fig. 6.4 Box plot of PASI scores before and after treatment. *Horizontal lines* Indicate the 75th percentile, median, and 25th percentile; *whiskers* indicate the range. From Grassberger and Hoch (2006)

Patients with no contraindication to UV exposure used a commercially available stand-up rapid-tan facility (UVA) for 3–5 min after each bath session. After UV exposure, the patients applied a generic skin lotion containing glycerine, Shea butter and *Aloe vera* extract. If the psoriasis significantly involved the scalp, then the patient's head was shaved before treatment.

As in the study by Özcelik et al. (2000), the primary efficacy outcome measure was the overall total reduction in PASI score and the proportion of patients with 50 % and 75 % improvement in PASI score (denoted as "PASI-50" and "PASI-75," respectively) at week 3, relative to baseline. The PASI-75 is the currently recognized benchmark of end-points used in psoriasis clinical trials. PASI-50 is also regarded as a clinically significant end-point (Carlin et al. 2004).

PASI scores were assessed using high-resolution digital color photographs taken at baseline and at the end of the 3-week treatment period. Baseline measurements were those made just prior to the beginning of treatment. Additionally, response to treatment was defined according to the rate of improvement in PASI score. Patient-reported outcomes were evaluated by a short questionnaire administered immediately after the 3-week course of treatment, and by a follow-up questionnaire sent to all patients 3–36 months after the study treatment. The follow-up questionnaire included questions concerning the duration of remission, the number of different treatment regimens prior to ichthyotherapy, the severity of a possible relapse, and the personal satisfaction with ichthyotherapy when compared to other treatments.

At the end of the 3-week treatment course, 31 of the 67 patients (46 %) achieved PASI-75 and an additional 30 patients (45 %) achieved PASI-50. For all patients, the average reduction in PASI score compared to baseline was 71.7 % ($P<0.0001$) (Figs. 6.4, 6.5, and 6.6). All of the patients experienced clinical improvement: complete resolution in three patients (4.5 %), marked improvement in 29 (43.3 %), moderate improvement in 29 (43.3 %) and slight improvement in 6 (8.9 %).

Assessment of patient-reported outcomes demonstrated substantial satisfaction with the treatment. The reported mean remission period was 8.58 months (95 % CI, 6.05–11.11). Overall, 87.5 % of patients reported a more favorable outcome with ichthyotherapy, compared to their previous therapies. This might be due, in part, to the

Fig. 6.5 Four patients suffering from psoriasis before (*left*) and after a 3-week course of treatment with ichthyotherapy (2 h daily fish bath) combined with UVA radiation (*right*)

Fig. 6.6 Three patients suffering from psoriasis before (*left*) and after a 3-week course of treatment with ichthyotherapy (2 h daily fish bath) combined with UVA radiation (*right*)

unusually long remission periods. Sixty-five percent of patients stated that subsequent relapses were less severe than before treatment. No significant adverse events were reported. Mild, transient bleeding from open crusted lesions was reported by one patient with eczema, and UV radiation-related erythema by two others.

In conclusion, only two small, non-controlled clinical studies have been published to date, but both studies show significant benefits of ichthyotherapy for psoriasis.

6.5 Impact on Health Related Quality of Life

Psoriasis, like many other disfiguring skin diseases, can negatively impact patients' health related quality of life (HRQoL) (Bhosle et al. 2006). Psoriasis patients often experience problems in body image and self-esteem, with feelings of shame and embarrassment regarding their appearance (Fortune et al. 2005). Krueger et al. (2001) reported that at least 20 % of psoriasis patients had once contemplated suicide. The chronic and recurring nature of this disease often results in feelings of hopelessness (Vardy et al. 2002). The lack of control over the disease may be one of the most bothersome issues for psoriasis patients (Rapp et al. 1998).

HRQoL is a well-established measure for evaluating treatment outcome in dermatology because HRQoL-questionnaires reflect patients' evaluation of the impact of disease and its treatment on their wellbeing. HRQoL is a physician-independent evaluation of treatment outcome. Many different validated measures and indices have been used in the past to assess HRQoL in psoriasis patients (Bhosle et al. 2006). The *Skindex-29* is a self-administered questionnaire with 29 items that assess three domains: burden of symptoms, social and physical functioning, and emotional response (Chren et al. 1996). The *Psoriasis Disability Index* is designed for use in adults (i.e. over the age of 16), and is comprised of 15 questions that assess daily activities, school or work, personal relationships, leisure, and treatment (Finlay and Kelly 1987).

In a recent study, presented at the *8th International Conference on Biotherapy* in Los Angeles evaluating health related quality of life in a cohort of 82 psoriasis patients before and after ichthyotherapy using "skindex-29" and the "Psoriasis Disability Index" (PDI), Grassberger (2010) reported a significant reduction in overall and subcategory scores in both indices (Figs. 6.7 and 6.8). The results of this HRQoL study further corroborate the results of the studies from Özcelik et al. (2000) and Grassberger and Hoch (2006): ichthyotherapy is, indeed, a highly efficacious treatment option with significant improvement in health related quality of life.

6.6 Ichthyotherapy for Congenital Ichthyosis – A Case Report

The male infant presented in this previously unpublished report was diagnosed with *lamellar ichthyosis* (*LI)*, which belongs to the *autosomal recessive congenital ichthyoses* (ARCI), characterized by non-bullous hyperkeratosis. LI has an estimated prevalence of 1:200,000–300,000 (Oji and Traupe 2006). Recommended treatments for infants with congenital ichthyosis include topical keratolytics (with propylene glycol and alpha hydroxyl), emollients with urea, calcipotriol (a derivative of calcitriol or vitamin D), tazarotene (a topical retinoid) and exceptionally oral retinoids (Vahlquist et al. 2008).

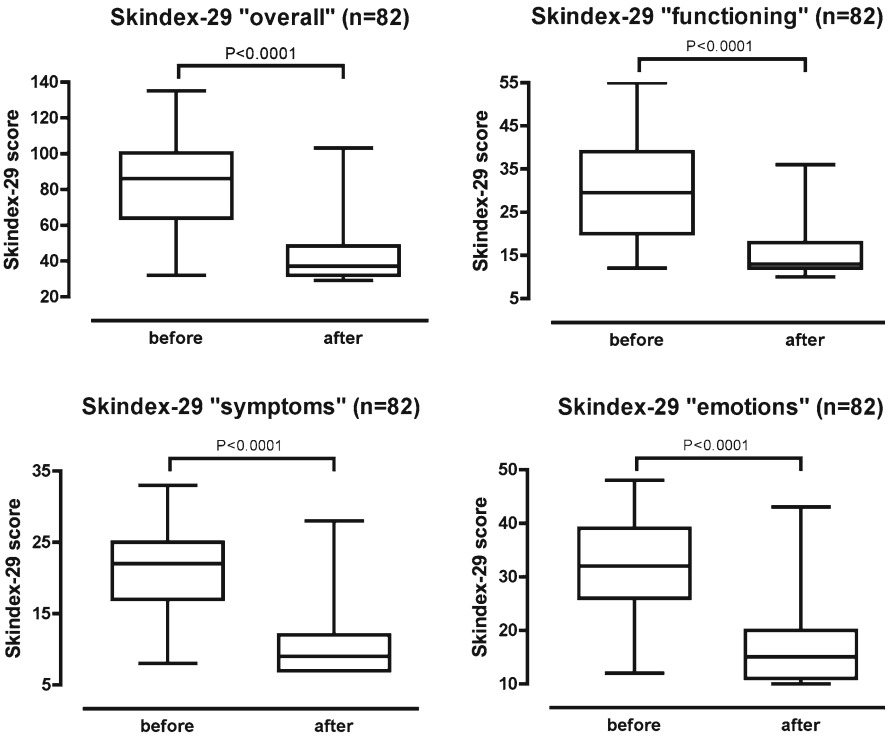

Fig. 6.7 Boxplot graphs for Skindex-29 before and after treatment for overall scores and the respective subcategories. *Horizontal lines* indicate the 75th percentile, median, and 25th percentile; *whiskers* indicate the range

Although the local side-effects of topical therapy for ichthyosis are usually minimal, patient compliance with the treatment regimen may still be poor. Topical treatment is time consuming, has to be repeated several times per day and can be quite painful. Creams or ointments may have unpleasant odors, might be difficult to apply, or might be rejected by the patient for other reasons (Vahlquist et al. 2008). As a result, these therapies are suboptimal and often fail.

6.6.1 Case

The caucasian male patient was born on term in 2001, encased in a tight shiny covering with erythroderma, referred to as collodion membrane (so-called "collodion baby"). During his first weeks of life, the membrane was gradually replaced with thick yellowish-brown plate-like hyperkeratotic scales on the whole integument, with marked palmoplantar hyperkeratosis. Soon after birth, he was diagnosed with congenital lamellar ichthyosis (Ichthyosis congenita, autosomal recessive). No known family anamnesis was detected. Subsequently the patient developed painful irregularly

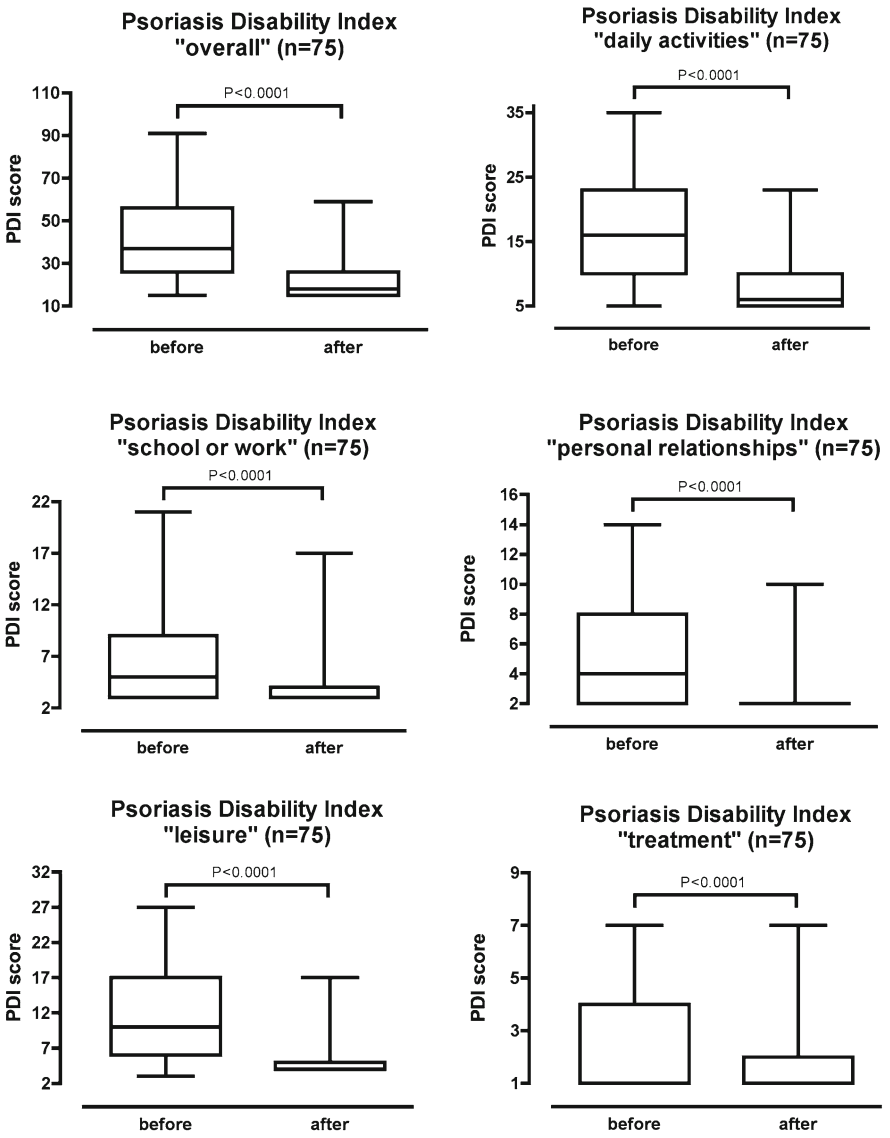

Fig. 6.8 Boxplot graphs for Psoriasis Disability Index before and after treatment for overall scores and the respective subcategories. *Horizontal lines* indicate the 75th percentile, median, and 25th percentile; *whiskers* indicate the range

branched deep cutaneous fissures with an inflammatory component causing severe pain on movement. An unpleasant and annoying smell emanated from the skin. The patient soon presented with ectropium, blepharoconjunctivitis and eclabium (Eversion of a lip). In the first year, contractures of the ischiocrural muscles and the peritrochanteric muscles ensued with already marked muscular atrophy (Fig. 6.9a).

Fig. 6.9 Successful long-term treatment of congenital ichthyosis with ichthyotherapy. (**a**) Before treatment; (**b**) during treatment in fish bath; (**c**) after several courses of ichthyotherapy; (**d**) through regular fish baths the young patient was able to elevate his arms and move without pain

By the time he reached 1.5 years, the scales had a thickness of up to 1 cm. Touching and moving his fingers was now very painful. A keratolytic treatment with Polidocanol and urea (Optiderm®), almond oil and chamomile was used on a daily basis. Pediatric assessment at age 2, documented a dystrophic toddler with massive delay in neuro-motor development.

In 2003, the child was admitted to a specialized clinic, where the patient received a complex treatment regimen of medical baths twice daily and several external ointments. The recommended regular mechanical keratolysis with a microfiber cloth was exceptionally painful and therefore very distressing for the mother as well. Regular physiotherapeutic/ergotherapeutic exercises were performed.

After discharge, the treatments were continued, but his condition soon worsened again. The child was again admitted to the specialized clinic. Instead of immersion baths, he now received a 15-min steam bath daily. Being essentially unable to sweat, this treatment was unbearable. Just 1 day without rubbing would result in an immediate relapse of symptoms.

Over the ensuing years, a variety of specialists was consulted without noticeable improvement. Movement was increasingly impaired, accordingly the child could not sit, hold a pencil or cutlery, bend, or raise his arms. In 2007, at age 6, the patient was scheduled for primary school, however, writing and prolonged sitting was a severe problem and made regular school attendance impossible.

In 2008, the child had his first ichthyotherapy treatment with specimens of *G. rufa* 2-h daily in the fish bath, for 3 weeks (Fig. 6.9b). After the first week, a rapid improvement was noted in the child's skin condition. After 2 weeks, almost all thick scales were removed (Fig. 6.9c), leaving only elbows and knees with some thicker scales. By now, the patient's life changed profoundly. Being able to move painlessly, the boy started to do things he never did before, e.g. using the slide and the carousel at the playground on his own, sitting, writing with a pencil, walking on his own, playing with a ball and raising his arms over the head (Fig. 6.9d). This resulted in joyful parents, a happy patient and a subsequent increase in body weight. By the end of treatment, even the head became virtually free of scales. The boy stated that he was able to sweat for the first time in his life, and was no longer getting overheated.

Yet, 5 days after the end of the 3-week treatment course, the child's skin conditions worsened again. As a result, continuous fish treatment was recommended. With the help of a public fundraising, a treatment tub was installed at the boy's home. Routine home treatment with fish baths every other day resulted in reduced redness, reduced itching, reduction of scales, and a resolution to the unpleasant and annoying odor of old, dead skin. This enormous increase in quality of life enabled the boy to visit school without handicap.

6.6.2 Case Discussion

Although systemic retinoids (which promote shedding of the hyperkeratotic plates) have become a standard therapeutic regime in disorders of keratinization (Lacour et al. 1996) the side-effects of systemic retinoids (alterations of lipids and liver

function tests) render them inadequate in infants. Successful treatment depends upon the continuous removal of the sick skin and must be done physically. Ichthyotherapy is an elegant and painless method of reducing the hyperkeratotic scales; consequently, quality of life is improved. This case highlights the benefits of ichthyotherapy with *G. rufa* on the quality of life in a young boy suffering from congenital lamellar ichthyosis.

6.7 Possible Mechanisms of Action

Several mechanisms have been suggested regarding the observed efficacy of ichthyotherapy. One obvious mechanism is the physical contact with the fish, which feed on the desquamating skin, thus leading to a rapid reduction of superficial skin scales. Additionally many patients consistently report a pleasing micro-massage like feeling while the fish nibble at their skin (Grassberger and Hoch 2006). Interestingly, the fish seem to prefer hyperkeratotic to healthy skin, possibly because it is easier to access and remove the scales (nibble).

Another suggested mechanism is the increased direct effect of ultraviolet radiation associated with ichthyotherapy, be it natural or in the form of a sunbed. Phototherapy is a well-recognized option for patients with widespread psoriasis lesions (Paul et al. 2012), and removal of scales by the fish probably facilitates greater penetration of UV rays into the dermis.

The presence of a high level of selenium (1.3 mg/L) in the Kangal hot springs water has also been suggested as a contributing factor (Özcelik et al. 2000), although Grassberger and Hoch (2006) reported similar efficacy with relatively low levels of selenium in the water. Therefore, water selenium concentration is probably not a major factor in the observed efficacy of ichthyotherapy.

Psychological factors like stress are regarded a causal or exacerbating factors in psoriasis (Arck and Paus 2006). Since most patients refer to the fish baths as relaxing and pleasing, the stress-reducing and psychological benefits of ichthyotherapy might contribute to the observed treatment benefits.

It is also possible that the oral secretions of the fish have direct anti-inflammatory or desquamation effects. The copious epidermal mucus of fish plays an important role in host defense, particularly in the prevention of colonization by pathogens. Several studies have shown that the surface mucus or "slime" of fish contains a variety of substances such as crinotoxins, calmodulin, pheromones and a variety of antimicrobial substances like fatty acids, immunoglobulins, complement components, lectins, lysozyme, proteolytic enzymes and antinociceptive substances (Subramanian et al. 2008; Jais et al. 1998). A polysaccharide isolated from the mucus of *Misgurnus anguillicaudatus*, a freshwater fish in the loach family (Cobitidae), exhibited strong anti-proliferative and apoptosis-inducing properties (Zhang and Huang 2005). This is a remarkable finding, given the fact that the problem in psoriasis and ichthyosis is a very high mitotic rate of epidermal cells. However, no research has been conducted yet with *G. rufa* to determine whether a similar effect exists in this species as well.

While various providers of ichthyotherapy claim on their websites that *G. rufa* secretes a unique enzyme called dithranol (synonym of anthralin, which prevents epithelial proliferation), there is no scientific evidence to support those claims. Actually, it seems highly unlikely that fish are capable of producing this substance, which is derived from the bark of the Araroba tree of South America and has been manufactured synthetically for decades.

6.8 Indications, Contraindications and Possible Side Effects

6.8.1 Indications

Probably all hyperkeratotic skin conditions would benefit from ichthyotherapy, since the mechanical removal of excessive skin scales by the fish accomplishes the most basic treatment endpoint for these disorders. As already demonstrated, psoriasis and one form of ichthyosis have been treated successfully (Özcelik et al. 2000; Grassberger and Hoch 2006; Grassberger 2010).

6.8.1.1 Psoriasis

Psoriasis is the illness that has most often been treated with ichthyotherapy. It is a common skin disorder with a worldwide distribution. The average prevalence in Europe and the USA has been estimated at about 2–3 %. It is hypothesized to be an immune-mediated disease with a genetic component. The patients often have extensive red and/or silver scaly plaques. These are areas of inflammation and excessive skin production, often on elbows, knees, scalp and genitals. Psoriasis is a chronic, often relapsing disease. Whilst considerable advances have been made in the management of this disease in recent years, there is still no cure, and no simple, safe and invariably effective treatment. The disease carries a substantial burden even when not extensive, and is associated with widespread treatment dissatisfaction.

6.8.1.2 Ichthyosis

Ichthyoses are a heterogeneous group of cornification disorders of the skin, characterized by generalized hyperkeratosis and excessive scaling (Oji et al. 2010). The small but diverse subgroup of *congenital ichthyoses* (CI), typically present at birth with a collodion membrane and/or ichthyosiform erythroderma. CI are very rare congenital disorders that often pose a diagnostic as well as a therapeutic challenge for the caring physician.

6.8.1.3 Diabetic Foot

Diabetic patients often suffer from xerosis and callus of the plantar skin, which often complicate the situation and predispose to the formation of plantar ulcers. In addition to optimal sugar control, effective prevention and treatment measures include pressure relief ("off-loading"), and avoidance or reduction of callus formation. Therefore, the treatment of calloused feet with ichthyotherapy in patients with diabetes mellitus might result in significant benefits. However, studies addressing this issue are still lacking, and special hygienic measures would have to be considered when these often immunocompromised patients are exposed to the potentially contaminated fish tank.

6.8.1.4 Atopic Dermatitis

Whether conditions like atopic dermatitis are also a suitable indication for this kind of alternative treatment remains to be studied.

6.8.1.5 Pedicure in "Fish-Spas"

In recent years, an increased number of "fish pedicure" salons opened to the public all over the world, where customers immerse their feet in small fish tubs to mid-calf level to let *G. rufa* (and in several cases other species) remove excess and thickened skin from their feet. In mid 2011, a survey among environmental health practitioners in the United Kingdom identified 279 registered fish spas in the UK alone (Health Protection Agency 2011). The fish nibble on the thickened skin of the feet with reported satisfaction of the customers. This application of *G. rufa* does not serve therapeutic purposes and is regarded as a wellness or recreational activity. One major problem associated with this new form of fish pedicure, according to local health authorities, is the possible spread of infections.

To educate fish pedicure salon operators on health and safety issues related to this practice, the British Health Protection Agency issued a manual for "guidance on the management of the public health risks from fish spas," based on available evidence and expert consensus (Health Protection Agency 2011). This manual was conceived and produced by representatives of the Health Protection Agency, Health Protection Scotland, the Health and Safety Laboratory and local authorities; it was agreed upon by the Department of Health, Social Services and Public Safety in Northern Ireland and Public Health Wales. The authors concluded that, on the evidence identified and the consensus view of experts, the risk of infection as a result of a fish pedicure is likely to be very low, but cannot be entirely excluded. In order to reduce the risk even further, the document provides operators of fish spas with practical recommendations for safe use.

Despite the fact that there is so far no scientific evidence for spread of infection through *G. rufa*, fish pedicures and ichthyotherapy facilities have been banned by

health authorities in several countries (including many U.S. states and Canadian provinces) because of safety concerns. These bans are mainly based on the following assumptions and circumstances: the fish used as "instruments" can neither be disinfected nor sterilized; animals (in this case fish) are prohibited from doctor's offices and pedicure salons; and animal welfare concerns, in general.

6.8.2 Contraindications

Based on general medical considerations, certain patient groups are likely to be at increased risk for infections when undergoing treatments like ichthyotherapy. Therefore, they should be discouraged from undergoing such treatment, especially if they have obvious breaks in the skin. Those at increased risk include severely immunocompromised patients or patients with immune deficiency, whether induced or due to an underlying illness.

Patients that have a known infection with a blood-borne virus, such as hepatitis B and C or HIV should be excluded from the treatment with fish reused on other patients. However, a home based treatment tub for single patient use can be an alternative in such cases. Generally, broken skin, bleeding wounds or infectious skin conditions are also considered contraindications. The transmissibility of contagious skin infections (i.e. bacterial skin infections, herpes viruses and warty human papilloma virus lesions) via ichthyotherapy is unknown, and at this point, individuals should not immerse active lesions into shared tanks in order to minimize potential risks to the fish and to other human clients.

The risk of contracting an infection can probably be reduced by use of disease-free fish reared in controlled facilities under high standards of husbandry and welfare (Verner-Jeffreys et al. 2012).

6.8.3 Side Effects

In the Turkish study by Özcelik et al. (2000), the only side effect reported was sunburn in two patients. In the first Austrian study by Grassberger and Hoch (2006), no severe side effects were recorded during the treatment period. Mild, transient bleeding from open crusted lesions was reported in one patient with eczema, and UV-radiation-related mild erythema in two others. Ichthyotherapy in combination with UVA-treatment was generally very well tolerated.

Given the scarcity of scientific studies on ichthyotherapy there is too little data to draw valid conclusions on possible side-effects and their frequency. Therefore, it seems prudent to consider the theoretical risks of infection when using living animals like fish for therapeutic purposes. However, based on available data and experience thus far, the observed benefits appear to far outweigh the minimal side-effects thus far encountered.

6.9 Hygienic Approach

6.9.1 Possible Risks

The major concern with ichthyotherapy is risk of infection. In the context of ichthyotherapy, there are three potential routes of pathogen transmission:

- fish to person (i.e. Zoonotic disease)
- water to person (i.e. Infection through contaminated water) and
- person to person (either via equipment or through the fish).

If the patient has an underlying condition with reduced defense mechanisms (i.e. immunocompromised patients) or open skin lesions the theoretical risk of infection is increased.

6.9.2 Transmission from Fish to Person (Zoonotic Disease)

Any disease or infection that is naturally transmissible from vertebrate animals to humans and vice-versa is classified as a zoonotic disease (zoonosis). In general, humans contract fish-borne bacterial diseases either through ingestion of contaminated fish tissue or water, or by injection of the organism into puncture wounds or abrasions. This mode of infection also includes transmission via fish tank surface or treatment tubs to patients.

6.9.2.1 Bacterial Infections

Exposures to fish-borne bacteria can result in asymptomatic or mild episodes of gastroenteritis or in localized infections of the skin and the underlying tissue (Nemetz and Shotts 1993). A few bacteria are highly pathogenic, and the status of the human host immune system plays a vital role in the severity of disease.

Gram-negative bacteria are the major cause of fish infectious diseases, both in terms of severity and incidence (Nemetz and Shotts 1993). A few of these microbes, along with some gram-positive species, also cause disease in humans (mostly of an opportunistic nature). According to Lehane and Rawlin (2000), the main pathogens acquired cutaneously from fish (through spine puncture or open wounds) are *Aeromonas hydrophila*, *Edwardsiella tarda*, *Erysipelothrix rhusiopathiae*, *Mycobacterium marinum*, *Streptococcus iniae*, *Vibrio vulnificus* and *Vibrio damsela*. The spectrum of manifestations is wide, varying from cases of mild cellulitis, to severe life-threatening necrotizing fasciitis requiring radical surgery, to sepsis and death (Finkelstein and Oren 2011).

In the setting of ichthyotherapy, the following organisms are probably of greatest concern: gram-positive bacteria like *Streptococcus* sp. and *Staphylococcus* sp., the latter causing erysipelas and cellulitis (a diffuse inflammation of connective tissue

with severe inflammation of the dermal and subcutaneous layers of the skin). *Erysipelothrix rhusiopathiae* and *Streptococcus iniae* are associated with handling fish, but zoonotic spread to humans occurs only rarely. *E. rhusiopathiae* can cause erysipeloid or "fish rose", with septicemia and endocarditis as potential sequel. *Streptococcus iniae* causes a high mortality rate and rapid death in infected fish. In humans, infection is clearly opportunistic with all cases to date associated with direct infection of puncture wounds during preparation of contaminated fish, generally in elderly or immunocompromised individuals (Agnew and Barnes 2007).

Mycobacteria like *Mycobacterium marinum*, *M. fortuitum*, *M. piscium* and *M. chelonei* can cause cutaneous lesions (so-called "fish tank granuloma" or "swimming pool granuloma") at skin contact sites, through contamination of lacerated or abraded skin, especially in immunocompromised patients. *M. marinum* has been identified in fish as well as in biofilms on inanimate surfaces (Aubry et al. 2002), and often infects home aquarium hobbyists. Infections with other non-tuberculous Mycobacteria (e.g. *M. mageritense*) have been reported in association with footbaths at nail saloons, and shaving prior to the footbaths has been identified as a risk factor (Winthrop et al. 2002; Gira et al. 2004). Since Mycobacteria are typically associated with pools and fish tanks, these pathogens and associated risk factors (e.g. broken skin and biofilms), deserve special attention in the ichthyotherapy setting.

Infections with gram-negative bacteria of the species *Plesiomonas shigelloides* cause gastroenteritis, followed by septicemia in immune deficient patients (Wadstrism and Ljungh 1991).

Aeromonas spp. such as *A. hydrophila*, *A. sobria* and *A. caviae* are capable of causing gastroenteritis and diarrheal disease when ingested, and localized wound infections when there is invasive contact with the skin, such as with water-related trauma (Janda and Abbott 2010). Interestingly, Aeromonas infections have been reported in association with the use of medicinal leeches (Snower et al. 1989; Whitaker et al. 2011), which also live in sweet waters. Skin infections may be superficial or may progress to cellulitis, deep muscle necrosis or septicemia, especially in the immunocompromised host. *Aeromonas sobria* has recently been reported in association with *G. rufa* by Majtan et al. (2012), however, reports of serious human infections are rare.

Other bacteria of relevance are *Pseudomonas* spp. and Enterobactericeae of the genera *Escherichia*, *Salmonella*, *Klebsiella and Edwardsiella.* Infection with *Edwardsiella tarda* is possible by ingestion or through a penetrating wound. Infected fish or contaminated water are possible sources of infection (Janda and Abbott 1993). A variety of disease may develop, including gastroenteritis, localized infections or septicemia. Salmonellae have also been reported in association with fish tanks and tropical fish.

Since most of these infections are associated with ingestion, patients should be educated to avoid swallowing the tub water and to wash their hands after the treatment or after hand contact with the fish.

6.9.2.2 Parasitic Infections

Fish parasites like *Diphyllobothrium latum* (fish tapeworm) pose no danger in well-controlled ichthyotherapy settings since the fish are only fed with commercially

available fish food in addition to the patient's skin scales. Therefore, the risk of fish tapeworm infestation can be ruled out, based on the absence of a suitable intermediate host (normally freshwater crustaceans). Fish flukes (trematodes in the phylum Platyhelminthes) can only be transmitted to man by eating raw or undercooked fish. Although potentially zoonotic species of *Giardia* and *Cryptosporidium* have been found in fish, there is no evidence that these could be transmitted via the mouths of *G. rufa*, nor via the water, as ingestion will not occur (Health Protection Agency 2011).

6.9.2.3 Viral and Fungal Infections

To the best of our knowledge, no human infections with fish specific viruses or fungi have been reported in the medical literature so far.

6.9.3 Transmission from Water to Person

In addition to the above-mentioned bacterial pathogens found in fish and fish-water, the following bacteria may be found in bathtub water not associated with fish: *Legionella pneumophila*, *Escherichia coli* and *Pseudomonas aeruginosa. Legionella pneumophila* can cause respiratory infections from mild to fatal pneumonia, but the risk of contracting a *Legionella*-associated respiratory tract infection is considered very low since substantial aerosols as in whirlpools and hot tubs are normally not generated during a fish bath. *Pseudomonas aeruginosa* is known to colonize biofilms on underwater surfaces and to cause whirlpool-associated dermatitis and folliculitis, which usually manifests as self-limiting pustular rash (Hudson et al. 1985; Ratnam et al. 1986; CDC 2000).

6.9.4 Transmission from Person to Person

In the ichthiotherapy setting, person-to-person spread of infection can occur either via direct patient contact, through the water, the equipment and/or the fish themselves. Health authorities are particularly concerned about the transmission of blood borne viruses that might occur as a result of sharing a fish bath.

6.9.4.1 Viral Infections

Although the risk of transmission of hepatitis B virus has been described as particularly high in athletes in contact and collision sports (Kordi and Wallace 2004) and survival of hepatitis B virus in the environment has been reported for 7 days on dry surfaces (Bond et al. 1981), there is no data available for survival in water. Ciesek

et al. (2010) found that at 37 °C and in a moist environment, hepatitis C virus was inactivated after just 2 days. It is generally considered that viral pathogens contaminating the fish's mouth are not likely to remain there in a sufficient manner to cause transmission. Heistinger et al. (2011), using a mammalian model virus, the Equine Herpes Virus 1 (EHV 1) as model for an enveloped DNA virus and Equine Rhino Virus (ERV) as model for a non-enveloped RNA-virus, have addressed this issue in *G. rufa*. Results showed that mammalian DNA virus did not survive on fish tissue, whereas mammalian RNA virus did survive on fish tissue for about 5 min. Although this study investigated only non-human viruses, there is no scientific record for cold-blooded animals like fish transmitting human viral disease, because viruses usually cannot survive in or on these animals. However, given the scarcity of publications, studies investigating the survival in fish and the possible transmission of blood-borne viruses are still important areas of future research.

Although the theoretical risk can never be completely excluded, and despite the lack of rigorous safety studies, it is noteworthy that the British Health Protection Agency (2011) has come to the conclusion that based on the available evidence to date, the risk of infection with a blood-borne virus as a result of a fish pedicure bath is likely to be extremely low.

6.9.4.2 Bacterial Infections

An infection with human pathogenic bacteria such as *Staphylococcus aureus* is more likely from skin contact with surfaces outside the water, with the risk being similar to that in a gym, since dilution by water makes water-borne transmission very unlikely (Health Protection Agency 2011).

6.9.4.3 Fungal Infections

Fungal infections like athlete's foot contracted via surviving organisms on surfaces such as floors and towels, are not unique to ichthyotherapy and are found frequently in areas like public pools, foot spas and sports clubs, where people walk barefoot. Following general standards of hygiene are therefore mandatory to prevent such infections.

6.9.5 Hygiene Guidelines for Ichthyotherapy

Apart from the guidance paper for fish foot spas by the British Health Protection Agency (2011), approved detailed hygiene standards for the treatment of skin diseases with ichthyotherapy have not yet been established. However, the variety and abundance of treatment centers and recent regulatory problems in many communities make it necessary to discuss possible health risks for patients and provide minimal requirements for hygiene when using this therapy. Based on the afore mentioned

theoretical modes of transmission, the following recommendations regarding maintenance of treatment tubs and fish should be followed as minimum requirements for ichthyotherapy facilities.

6.9.5.1 Hygienic Measures

In light of the potential risks of infections, predominantly from bacterial pathogens, the most important steps to establish a hygienic and safe ichthyotherapy or fish spa are:

- the use of an open freshwater system, which renews the bathing water constantly or at regular intervals, with an estimated complete water exchange 3–4 times a day,
- preventing floating debris and contaminants through the use of a cascade filters or similar physical barriers,
- use of a filter pump to constantly clean the recycling water, thereby removing fish excrements and immersed particles,
- use of an ultraviolet (UV-C) water sterilization device,
- oxygen enrichment of the water and
- use of an oxygen-separating water and surface disinfectant that is approved for use in aquaculture.

Ideally, all of the above-mentioned measures are taken by a fully automated digitally controlled system.

Plants, rocks or sand in the treatment tubs are neither necessary nor appropriate, since these features are prone to biofilm formation. However, corresponding to the fish's natural habitat, the floor of the tubs should provide hiding places for the fish to comply with animal welfare requirements.

6.9.5.2 Water Disinfection

Unlike other pool facilities, the water in the treatment tubs cannot be treated with classical chemical disinfectants, e.g. chlorine or quaternary ammonium compounds, since these substances are not tolerated by the fish. Therefore, an oxygen separating substance, approved for use in aquaculture (e.g. Sanosil®) has proven useful in disinfecting the water to the required level, whilst maintaining fish viability. The active substances of Sanosil® are stabilized hydrogen peroxide, and traces of ionic silver. Both substances work synergistically and are highly effective against a broad spectrum of viruses, bacteria and fungi, are active against biofilm formation and – in the correct concentrations – are non-toxic to patients and fish.

6.9.5.3 Surface Disinfection

Most common nosocomial pathogens may well survive and persist on inanimate surfaces for weeks or even months, making these surfaces a continuous source of

transmission, if no regular preventive surface disinfection is performed (Kramer et al. 2006). Therefore, general guidelines for surface disinfection in specific patient care areas, apart from the treatment tubs, are mandatory for ichthyotherapy facilities. The treatment tubs should be thoroughly cleaned and disinfected at regular intervals with an oxygen-separating agent, preferably after each 3-week treatment course.

6.9.5.4 Water and Fish Samples

For surveillance of microbiological parameters, water samples should be drawn at regular intervals to monitor quality. Official regulations from local health authorities for water quality in public pools or whirlpools may be followed as guidance and should provide a minimal framework. To control potential zoonotic infections, scheduled fish sampling by accredited veterinarian specialists is recommended, with special focus on the presence of non-tuberculous Mycobacteria and *Aeromonas* spp. A fish health certificate should always be obtained.

6.9.5.5 Patient Requirements

We recommend that patients should be tested for blood-borne viruses (BBV) such as HIV and Hepatitis B and C prior to fish treatment, since many people are unaware of their BBV status. Additionally, patients should be under medical supervision throughout the entire course of treatment, and should be excluded from ichthyotherapy when an infectious skin disease is suspected or diagnosed (see also "contraindications"). After providing patients with all relevant information on ichthyotherapy, including contraindications and possible side-effects, they should be asked to sign an informed consent form prior to treatment, to ensure that they understood the information given and are not aware of any contraindications to the treatment.

In addition to the above recommendations, one must always comply with the local regulations when performing ichthyotherapy.

6.10 Practical Application

6.10.1 Fish Species

The few scientific studies published so far employed the species *G. rufa.* Therefore, no other fish should be used for this treatment unless safety and efficacy data are available for those species. The fish, preferably reared under veterinary supervision in an adjacent breeding facility (Fig. 6.10a), should be at least 3 cm in length

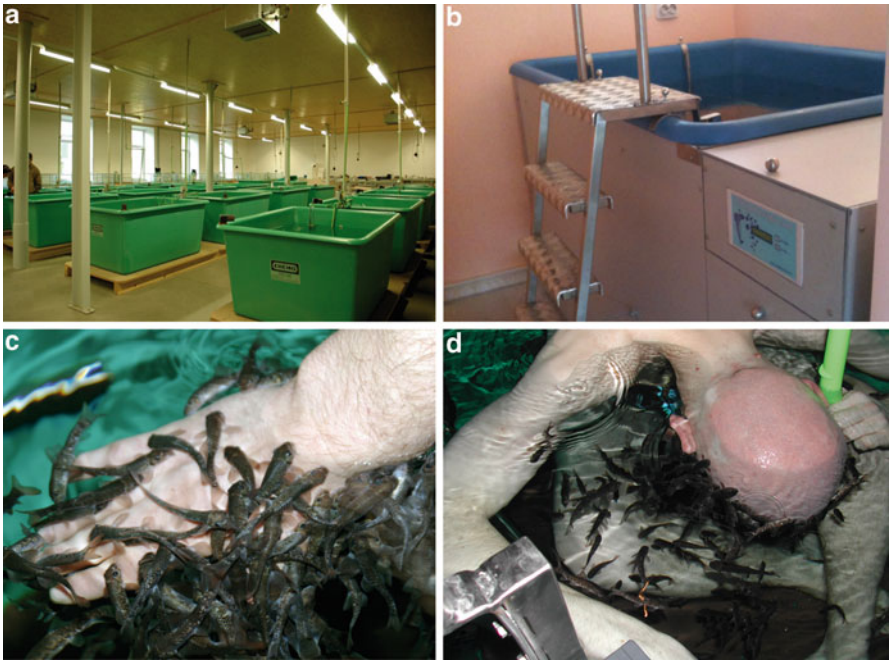

Fig. 6.10 (**a**) In conjunction with an ichthyotherapy facility, a larger scale adjacent breeding facility is necessary, especially when home users with their own tubs have to be supplied; (**b**) treatment tub with built in fully automated control unit; (**c**) healthy fish are a prerequisite for a successful treatment; (**d**) in some cases special procedures are necessary, i.e. shaving the head and using a snorkel to treat scalp lesions

(approx. 1.5 years old) when used for treatment. The fish must always be fed nutritious (i.e. commercially available) fish food daily after the treatment sessions, since feeding on patients' skin scales does not provide most of the required nutrients. There should never be any shortness in food supply and it is not necessary to starve the fish in order to be therapeutically active.

6.10.2 Tubs and Number of Fish

Every patient should be allocated his own treatment cabin equipped with a tub. The treatment tubs, made from food-safe plastic usually have a capacity of 700–1,000 l (Fig. 6.10b). Between 250 and 400 fish are used on each patient, depending on the size and severity of the skin lesions. Ideally, the bath tubs are equipped with an elaborate hygiene and fresh water system, to ensure healthy active fish (Fig. 6.10c) and patient safety. The bath tubs should be set to a comfortably warm temperature (about 36 °C).

6.10.3 Duration of Treatment

According to Grassberger and Hoch (2006) a treatment course with a duration of approximately 3 weeks with a daily 2-h fish bath is sufficient for a satisfying result in psoriasis. Ongoing treatments might be necessary thereafter to maintain the treatment result and keep the patient symptom free, i.e. in remission. Daily treatments lasting for 1–2 h makes ichthyotherapy acceptable for most patients, and considerably improves compliance (Grassberger and Hoch 2006).

6.10.4 UV-Radiation (Phototherapy)

Patients with no contraindication to UV exposure may additionally use a commercially available stand-up tan facility after each bath session according to skin type and after consultation with a dermatologist.

6.10.5 Emollients

In the Austrian ichthyotherapy study (Grassberger and Hoch 2006), patients applied a generic skin lotion (emollient) containing Shea butter and *Aloe vera* extract after fish and UV exposure. Whether this is a major contribution to the observed effect remains to be studied.

6.10.6 Special Procedures

If psoriasis lesions severely involve the scalp, the patient's head can optionally be shaved before treatment and then immersed while the patient breathes through a snorkel (Fig. 6.10d). Sensitive skin areas might be clothed or taped to avoid irritation. However, it should be emphasized that bleeding skin lesions present a contraindication in most treatment centers.

6.11 Future Research

6.11.1 Efficacy

Based on the few but well documented clinical studies, ichthyotherapy combined with a short course of UVA treatment can be regarded as a relatively safe and effective treatment option for patients with disfiguring hyperkeratotic skin conditions like

psoriasis and ichthyosis. Prospective randomized controlled trials are now warranted to validate the efficacy of this unusual and apparently highly effective biotherapeutic modality, and to compare ichthyotherapy with controls (e.g., the conventional standard regimens or water with the UV therapy alone). Additionally, the aesthetic or recreational use of *G. rufa* in the form of foot spas and pedicure parties should be clearly separated from the medical or therapeutic application of these fish.

6.11.2 Hygiene

Based on the above given hygienic standards, "Best Practice Guidelines" regarding minimal requirements in hygiene should be established and subsequently published to provide health authorities with an aid to assess commercial ichthyotherapy facilities and to harmonize treatment standards throughout the world. Larger safety studies might uncover some uncommon side effects, or might help to allay fears that serious problems will soon declare themselves. Although no experiment can prove that an event will never occur, laboratory models can help demonstrate the likelihood (or unlikelihood) of various theoretical infectious complications. Given the regulatory restrictions that currently exist in many countries, and given the large numbers of people who could potentially benefit from ichthyotherapy if it were more widely available, such studies will certainly be undertaken in the near future.

6.11.3 Mechanisms of Action

The underlying mechanisms of ichthyotherapy are not yet fully understood. The most noticeable effect of the fish is removal of excess skin scales. However, the observed dramatic reduction of the inflammatory component in psoriasis patients suggests additional, possibly molecular mechanisms. Biochemical studies should be undertaken to identify and characterize the properties of *G. rufa* mucus in this context.

References

Abdoli A (2000) The inland freshwater fishes of Iran (in: Farsi). Iranian Museum of Natural and Wildlife, Tehran, 378 pp

Agnew W, Barnes AC (2007) *Streptococcus iniae*: an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. Vet Microbiol 122(1–2):1–15

Arck P, Paus R (2006) From the brain-skin connection: the neuroendocrine-immune misalliance of stress and itch. Neuroimmunomodulation 13(5–6):347–356

Aubry A, Chosidow O, Caumes E, Robert J, Cambau E (2002) Sixty-three cases of *Mycobacterium marinum* infection. Arch Intern Med 162:1746–1752

Berg LS (1949) Freshwater fishes of Iran and adjacent countries. Tr Zool Inst Akad Nauk SSSR 8:783–858

Bhosle MJ, Kulkarni A, Feldman SR, Balkrishnan R (2006) Quality of life in patients with psoriasis. Health Qual Life Outcomes 6(4):35. doi:10.1186/1477-7525-4-35

Bond WW, Favero MS, Petersen NJ, Gravelle CR, Ebert JW, Maynard JE (1981) Survival of hepatitis B virus after drying and storage for one week. Lancet 1(8219):550–551

Carlin CS, Feldman SR, Krueger JG, Menter A, Krueger GG (2004) A 50 % reduction in the Psoriasis Area and Severity Score (PASI 50) is a clinically significant endpoint in the assessment of psoriasis. J Am Acad Dermatol 50:859–866

CDC (2000) Pseudomonas dermatitis/folliculitis associated with pools and hot tubs – Colorado and Maine 1999–2000. MMWR 49(48):1087–1091

Chren MM, Lasek RJ, Quinn LM, Mostow EN, Zyzanski SJ (1996) Skindex, a quality-of-life measure for patients with skin diseases: reliability, validity and responsiveness. J Invest Dermatol 107:707–713

Ciesek S, Friesland M, Steinmann J, Becker B, Wedemeyer H, Manns MP, Steinmann J, Pietschmann T, Steinmann E (2010) How stable is the hepatitis C virus (HCV)? Environmental stability of HCV and its susceptibility to chemical biocides. J Infect Dis 201(12):1859–1866

Coad BW (2012) Freshwater fishes of Iran. http://www.briancoad.com. Accessed 29 Oct 2012

Finkelstein R, Oren I (2011) Soft tissue infections caused by marine bacterial pathogens: epidemiology, diagnosis, and management. Curr Infect Dis Rep 13(5):470–477

Finlay AY, Kelly SE (1987) Psoriasis: an index of disability. Clin Exp Dermatol 12:8–11

Fishbase.org "*Garra rufa* (Heckel, 1843)" http://www.fishbase.org/summary/Garra-rufa.html. Accessed 29 Oct 2012

Fortune DG, Richards HL, Griffiths CE (2005) Psychologic factors in psoriasis: consequences, mechanisms, and interventions. Dermatol Clin 23:681–694

Gira AK, Reisenauer AH, Hammock L, Nadiminti U, Macy JT, Reeves A, Burnett C, Yakrus MA, Toney S, Jensen BJ, Blumberg HM, Caughman SW, Nolte FS (2004) Furunculosis due to *Mycobacterium mageritense* associated with footbaths at a nail salon. J Clin Microbiol 42(4):1813–1817

Grassberger M (2010) Ichthyotherapy for skin disease. Presented at the 8th international conference on biotherapy, Los Angeles, 11–14 Nov 2010

Grassberger M, Hoch W (2006) Ichthyotherapy as alternative treatment for patients with psoriasis: a pilot study. Evid Based Complement Alternat Med 3(4):483–488

Health Protection Agency (2011) Guidance on the management of the public health risks from fish pedicures. http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317131045549. Accessed 25 Oct 2012

Heistinger K, Heistinger H, Lussy H, Nowotny N (2011) Analysis of potential microbiological risks in Ichthyotherapy using Kangal fish (*Garra rufa*). Egypt J Aquat Biol Fish 15(3):525–537

Hudson PJ, Vogt RL, Jillson DA, Kappel SJ, Highsmith AK (1985) Duration of whirlpool-spa use as a risk factor for Pseudomonas dermatitis. Am J Epidemiol 122(5):915–917

Jais AM, Matori MF, Kittakoop P, Sowanborirux K (1998) Fatty acid compositions in mucus and roe of Haruan, *Channa striatus*, for wound healing. Gen Pharmacol 30(4):561–563

Janda JM, Abbott SL (1993) Infections associated with the genus *Edwardsiella*: the role of *Edwardsiella tarda* in human disease. Clin Infect Dis 17(4):742–748

Janda JM, Abbott SL (2010) The genus Aeromonas: taxonomy, pathogenicity, and infection. Clin Microbiol Rev 23(1):35–73

Kordi R, Wallace WA (2004) Blood borne infections in sport: risks of transmission, methods of prevention, and recommendations for hepatitis B vaccination. Br J Sports Med 38(6): 678–684

Kramer A, Schwebke I, Kampf G (2006) How long do nosocomial pathogens persist on inanimate surfaces?A systematic review. BMC Infect Dis 6:130

Krueger G, Koo J, Lebwohl M, Menter A, Stern RS, Rolstad T (2001) The impact of psoriasis on quality of life: results of a 1998 National Psoriasis Foundation patient-membership survey. Arch Dermatol 137:280–284

Kullander SO, Fang F (2004) Seven new species of *Garra* (Cyprinidae: Cyprininae) from the Rakhine Yoma, southern Myanmar. Ichthyol Explor Freshw 15(3):257–278

Lacour M, Mehta-Nikhar B, Atherton DJ, Harper JI (1996) An appraisal of acitretin therapy in children with inherited disorders of keratinization. Br J Dermatol 134(6):1023–1029
Lehane L, Rawlin GT (2000) Topically acquired bacterial zoonoses from fish: a review. Med J Aust 173(5):256–259
Majtan J, Cerny J, Ofukana A, Takac P, Kozanek M (2012) Mortality of therapeutic fish *Garra rufa* caused by Aeromonas sobria. Asian Pac J Trop Biomed 2(2):85–87
Menon AGK (1964) Monograph of the cyprinid fishes of the genus *Garra* Hamilton. Mem Indian Mus 14:173–260
Nemetz TG, Shotts EB (1993) Zoonotic diseases. In: Stoskopf MK (ed) Fish medicine. WB Saunders Company, Philadelphia, pp 214–220
Oji V, Traupe H (2006) Ichthyoses: differential diagnosis and molecular genetics. Eur J Dermatol 16(4):349–359
Oji V, Tadini G, Akiyama M, Blanchet Bardon C, Bodemer C, Bourrat E, Coudiere P, DiGiovanna JJ et al (2010) Revised nomenclature and classification of inherited ichthyoses: results of the First Ichthyosis Consensus Conference in Sorèze 2009. J Am Acad Dermatol 63(4): 607–641
Özcelik S, Polat HH, Akyol M, Yalcin AN, Ozcelik D, Marufihah M (2000) Kangal hot spring with fish and psoriasis treatment. J Dermatol 27(6):386–390
Paul C, Gallini A, Archier E, Castela E, Devaux S, Aractingi S, Aubin F, Bachelez H, Cribier B, Joly P, Jullien D, Le Maître M, Misery L, Richard MA, Ortonne JP (2012) Evidence-based recommendations on topical treatment and phototherapy of psoriasis: systematic review and expert opinion of a panel of dermatologists. J Eur Acad Dermatol Venereol 26(Suppl 3):1–10
Rapp SR, Feldman S, Fleischer AB Jr, Reboussin DM, Exum ML (1998) Health related quality of life in psoriasis: a biopsychosocial model and measures. In: Rajagopalan R, Sherertz EF, Anderson R (eds) Care management of skin diseases life quality and economic impact. Marcel Dekker, New York, pp 125–145
Ratnam S, Hogan K, March SB, Butler RW (1986) Whirlpool-associated folliculitis caused by *Pseudomonas aeruginosa*: report of an outbreak and review. J Clin Microbiol 23(3):655–659
Snower DP, Ruef C, Kuritza AP, Edberg SC (1989) *Aeromonas hydrophila* infection associated with the use of medicinal leeches. J Clin Microbiol 27(6):1421–1422
Subramanian S, Ross NW, MacKinnon SL (2008) Comparison of antimicrobial activity in the epidermal mucus extracts of fish. Comp Biochem Physiol B Biochem Mol Biol 150(1):85–92
Teimori A (2006) Preliminary study of freshwater fish diversity in Fars province. M.Sc. thesis, Shiraz University, Shiraz
Teimori A, Esmaeili HR, Ansari TH (2011) Micro-structure consideration of the adhesive organ in Doctor Fish, *Garra rufa* (Teleostei; Cyprinidae) from the Persian Gulf Basin. Turk J Fish Aquat Sci 11:407–411
Timur M, Çolak A, Marufi M (1983) A study on the systematic identification of the Balikli thermal spring (Sivas) fish and the curative effects of the fish on dermal diseases. Vet Fakült Dergisi (Ankara) 30:276–282
Vahlquist A, Gånemo A, Virtanen M (2008) Congenital ichthyosis: an overview of current and emerging therapies. Acta Derm Venereol 88:4–14
Vardy D, Besser A, Amir M, Gesthalter B, Biton A, Buskila D (2002) Experiences of stigmatization play a role in mediating the impact of disease severity on quality of life in psoriasis patients. Br J Dermatol 147:736–742
Verner-Jeffreys DW, Baker-Austin C, Pond MJ, Rimmer GSE, Kerr R, Stone D et al (2012) Zoonotic disease pathogens in fish used for pedicure [letter]. Emerg Infect Dis 18(6):1006–1008, http://dx.doi.org/10.3201/eid1806.111782
Wadstrism T, Ljungh A (1991) *Aeromonas* and *Plesiomonas* as food and waterborne pathogens. Int J Food Microbiol 12:303–312
Warwick D, Warwick J (1989) The doctor fish – a cure for psoriasis? Lancet 335:1093–1094
Whitaker IS, Josty IC, Hawkins S, Azzopardi E, Naderi N, Graf J, Damaris L, Lineaweaver WC, Kon M (2011) Medicinal leeches and the microsurgeon: a four-year study, clinical series and risk benefit review. Microsurgery 31(4):281–287

Winthrop KL, Abrams M, Yakrus M, Schwartz I, Ely J, Gillies D, Vugia DJ (2002) An outbreak of mycobacterial furunculosis associated with footbaths at a nail salon. N Engl J Med 346(18):1366–1371

Zhang CX, Huang KX (2005) Apoptosis induction on HL-60 cells of a novel polysaccharide from the mucus of the loach, *Misgurnus anguillicaudatus*. J Ethnopharmacol 99(3):385–390