The base became operational when the No 9 Squadron transferred from RAAF Point Cook, Victoria, to Rathmines, with Seagull flying boats. Catalina flying boats arrived at the base in February 1941 and, by September 1943, the base comprised 14 Catalinas, two Seagulls, a Dornier and a Dolphin.

Rathmines was an important base for the Catalina flying boats and their squadrons, which played a significant role in Australia's RAAF defensive operations during the Second World War. The RAAF operated 168 between 1941 and 1950, flown by four front line squadrons, two communications units and three air-sea rescue flights during the war. The Catalina flying boats were the only aircraft to see service with the RAAF for the total wartime operations against Japan.

Catalina operations included reconnaissance bombing, mine laying, supplying troops, coast watches and air-sea rescue missions. RAAF Catalinas were famous for their precision laying of mines in enemy water ways and harbours. The Catalina flying boat was one of the durable and effective aircraft of the war, due to their range, endurance and good load carrying capacity. Consequently, they were used by almost all the Allied services including the Royal Air Force and RAAF. Although it was one of the slowest combat aircraft of the war, it outsold all the newer, faster and better-equipped replacements of other manufacturers. Flying boats such as the Catalina placed a special demand on training air crews who not only learnt to fly the aircraft but needed to learn manoeuvres in sea conditions which was usually associated with naval operations. The famous Black Cats were used on covert night operations mine laying just about every enemy port in the South West Pacific Area, operations extending as far as the Chinese coast. During these operations, 322 aircrew were lost. Catalinas and air crews from Rathmines were involved in the defence of Australia in war events such as the Battle of the Coral Sea. In 1942, a Japanese taskforce bound for Port Moresby was located and followed by Catalina aircraft. The extensive duration of the Catalina enabled the aircraft to remain in contact with the Japanese force and call in the navy. Reports transmitted from the aircraft allowed American and Australian navies to intercept the Japanese force, resulting in the Battle of the Coral Sea. Flying boats from Rathmines were a part of this battle, which ended with the Japanese forced to withdraw from Australian waters, effectively ending the immediate threat of a Japanese invasion of the Australian mainland.

The base was also involved with the mining of Manila Harbour, which involved 24 RAAF Catalinas, eight of which were from No 11 Squadron aircraft which originated from Rathmines.

The Rathmines Base was an important flying boat crew training facility for the war. The base housed the Operational Training Unit for Catalina aircrew. During the 1940s, crews for No.s 9, 10, 11 Squadrons trained at Rathmines, with a total of over 200 Catalina air crews trained at the base. The base also provided a Flying Boat Repair Depot and a Marine Section Repair Depot. New flying boats were made in the US, and were then converted at Rathmines for operational duties.

The base reached its peak strength of almost 3,000 officers and other ranks in 1944 – 45, and was the largest flying boat base in Australia.